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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF PHYSICS AND CHEMISTRY

THE HEAVY NUCLEI OF PRIMARY COSMIC RADIATION*

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Introduction

Our ideas as to the nature of primary cosmic radiation have been revised radically and recurrently during the past few decades. About thirty years ago it was widely supposed that this radiation consists of penetrating electromagnetic waves. Shortly thereafter it was established that the primaries, or at least, a large proportion of them, must be charged particles (J.Clay, 1928; A. H. Compton, 1933), and these were thought to be electrons. Later this concept became suspect (T. H. Johnson, 1938), and it was shown that the charged particles are protons rather than electrons (M. Schein *et al.*, 1941). Ten years ago it was discovered that, in addition to protons, the primary cosmic radiation also consists of heavier nuclei, from those of helium up to nuclei more massive than those of iron (P. Freier *et al.*, 1948a).

During the past decade this picture has changed little in a qualitative way, although we have acquired quantitative information about the absolute intensities and the chemical composition of the primaries, that is, their relative abundances. Long before the nature of the primaries was established, it was already realized that they possess characteristic energies of billions of electron volts (Bev). This led to Millikan's hypothesis that cosmic rays originate in the annihilation of atoms in space. However, as K. K. Darrow aptly put it, while cosmic rays were once thought to be the death cries of the elements, they now turn out to be the elements themselves. They are, in fact, atoms stripped of their electrons and moving with velocities close to that of light.

Where do these nuclei originate, and how do they achieve their prodigious velocities? These are questions of deep cosmologic significance, to which we have as yet no definitive answers. A principal goal of current cosmic ray research is to learn more about the character of these high-energy particles that reach the vicinity of the earth and bombard the top of our atmosphere. This information can then be checked against various theories of origin and acceleration. Available evidence suggests that most, if not all, of the cosmic rays that strike the earth originate in our own galaxy. A small fraction of the radiation comes from the sun. It is possible, but difficult to establish, that a minute part of the radiation – including the most energetic particles yet detected – has an extra-

*This paper, illustrated with slides, was presented at a meeting of the Section on May 6, 1958.

galactic origin. Recent astrophysical observations have revealed the fact that the remarkable physical conditions prevailing in certain celestial objects, such as the Crab nebula, make them strongly suspect as sites of origin for the cosmic radiation. However, I shall deal only indirectly with these fascinating questions.

The subject of this paper is the heavy nuclei of the primary cosmic radiation. I shall first examine the methods available for detecting and identifying these nuclei, and for estimating their virgin flux at the top of the atmosphere. Second, I shall endeavor to summarize some of the salient information about these heavy nuclei. Third, I shall discuss a few of the principal problems that remain to be solved and, finally, I shall indicate the prospects of extending our knowledge with the help of ultrahigh-altitude balloon and rocket flights.

Methods of Detection and Identification

In order to obtain the required experimental information about the heavy primary nuclei (HPN), one needs suitable detectors that can record and identify the particles. Moreover, one must send these instruments to very high altitudes, and keep them there a sufficient time to accumulate the necessary data. It is well known that cosmic radiation is detectable throughout the atmosphere and, indeed, at considerable depths underground. Nevertheless, even at mountain and airplane altitudes the radiation is already quite different from that which strikes the top of the atmosphere. Consequently, to explore the nature of the primaries, it is necessary to attain the highest possible altitudes, since the primary radiation is rapidly transformed in its passage through the uppermost layers of air. This is true of the abundant hydrogen component, and it is especially true of the heavier nuclei. Collisions with nitrogen and oxygen nuclei break up these primaries and create showers of new particles that cascade down through the atmosphere, generating secondaries, tertiaries, and more remote progeny.

The chance of collisions is high compared with that for protons: the geometric cross sections of the heavy nuclei are large, and their collision mean free paths correspondingly short, of the order of 25 gm./cm.^{2} The HPN that survive collisions are soon arrested by ionization loss. For a nucleus of atomic number Z the range is reduced by approximately the factor $2/Z$ with respect to that of a proton having the same velocity. Thus, an incoming Mg nucleus that escapes collision is brought to rest in a path one sixth as long as that required by a proton having the same energy per nucleon. As a result of both of these processes, nuclear collision and ionization loss, very few of the heavy nuclei penetrate to depths as great as 0.1 atmosphere. In order, however, to have a reasonable chance of deducing the true primary distribution in abundance of the heavy elements, one must attain pressure altitudes of approximately 10 gm./cm.^{2}, that is, 0.01 atmosphere.

Large plastic balloons – so-called constant-volume balloons – for achieving these heights have been available for nearly a decade (FIGURE 1). Among those recently flown are some balloons with fully inflated volumes of one to three million cubic feet. They can carry substantial payloads up to altitudes exceeding 30 km. and can stay at a level height

FIGURE 1. Polyethylene balloon launched from the deck of a ship to collect data on the cosmic radiation at altitudes > 100,000 feet. In the stratosphere, the bubble of helium gas expands and fills the balloon until the latter becomes nearly spherical.

for many hours.* Rockets have also been employed, and these can reach even greater altitudes, but hitherto their flights have been limited to a duration of several minutes. To accumulate a statistically significant amount of material, it is necessary to have longer exposures.

Next we consider the problems of detection and identification. As in nuclear physics generally, the phenomenon most useful for detection is the ionization produced along the path of the particle. We recall that the space rate of energy loss by ionization for a particle carrying charge Z can be written:†

$$I \sim Z^2 f(v)$$

For a given velocity, particles with $Z > 1$ will ionize more densely than singly charged particles. This will be true of the heavy nuclei even when they ionize at or near their minimum rates, that is, at relativistic velocities. For example, a relativistic lithium nucleus ionizes 9 times as densely, and carbon 36 times as densely, as a relativistic proton. This distinctive property of the heavier nuclei – that they leave relatively dense tracks at *all* energies – makes them easier to detect.

However, these particles cannot in general be identified by their ionization alone. We can see why this is true by reference to FIGURE 2, in which the rate of ionization loss I is plotted for particles with different charges Z against a function of the velocity – the kinetic energy expressed in rest-mass units. At sufficiently high velocities, for which the kinetic

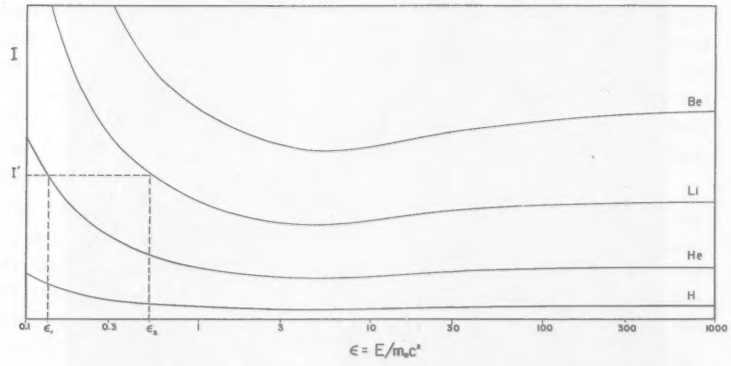

FIGURE 2. Space rate of energy loss by ionization I versus kinetic energy ϵ expressed in rest-mass units for nuclei of the four lightest elements. An He nucleus with energy ϵ_1 produces as dense a track as a Li nucleus having energy ϵ_2.

*A flight can last considerably longer, particularly if one is not concerned about recovery.

†The function $f(v)$ can be approximated, over a wide range of velocities, by $1/v^2$.

energy of the particle exceeds ~ 2 rest masses, these curves become relatively flat.* It can be seen from the diagram that slow particles with low Z can ionize as heavily as faster particles having high Z. Thus, the same rate of ionization loss I', shown by the horizontal dashed line, can characterize a helium or lithium nucleus having respective energies ϵ_1 and ϵ_2. However, for a given value of I, the heavier nuclei have ranges that greatly exceed those of hydrogen and mean scattering angles that are much smaller. Thus, under conditions where the heavy particles are known to be "fast" (for example, $\epsilon > 1.5$ rest masses), the problem is considerably simplified, for then they must be differentiated only from slow, singly charged particles. This introduces an important simplification in the investigation of the HPN at sufficiently low geomagnetic latitudes, where the magnetic field of the earth admits only particles with relativistic velocities.

Among the track characteristics (in addition to ionization) which are used for identification as well as energy estimation are the following: (1) the range (R), when the energy of the particle is sufficiently low so that it can be arrested by ionization within the detecting apparatus; for even if it leaves the detector without coming to rest, it may be possible to measure the change of ionization with distance along the path of the particle; (2) the multiple coulomb scattering (S) suffered by the charged particle along its path; and (3) the Cerenkov radiation emitted by a sufficiently fast particle.

Many types of detectors have been used to study the heavy primary nuclei. Among these are ionization chambers, "inefficient" counters (relatively insensitive to weakly ionizing particles), proportional counters, scintillation counters, Cerenkov detectors, and cloud chambers. Combinations of several of these, for example counter telescopes, have been applied. All these instruments have furnished data on the heavy primary component. However, the detector primarily responsible for the discovery of this component, the one that has been employed most extensively and has contributed the most to our knowledge of the HPN, is the photographic "nuclear" emulsion.† This detector is less hampered by the rigorous limitations of high-altitude balloon flights than are other instruments. The latter are generally heavier, more complicated, and more fragile than a stack of emulsion that requires a minimum of auxiliary instrumentation. In the sensitive emulsion, with its high stopping power, a moving ion leaves a signature of microscopic silver grains and blobs. Each millimeter of path length contains a wealth of information. Moreover, a reasonable-sized stack of stripped emulsions can accumulate more

*Actually the theory of ionization loss of relativistic particles predicts that each of these curves reaches a minimum that is followed at higher particle energies by a rise in the rate of ionization loss. The latter effect is ignored in the present discussion.

†For a discussion of emulsion techniques the reader is referred to the article on this subject in the new edition of *Handbuch der Physik* (M. M. Shapiro, 1958).

statistics on the primaries in a single flight than other detectors. With suitable alignment of the "pellicle layers" in a stripped emulsion stack it is possible readily to follow the tracks of heavy nuclei from layer to layer of the dense medium until sufficient path length has been measured for the purpose at hand. The various parameters discussed above, with the exception of Cerenkov radiation, can all be exploited in nuclear emulsion under appropriate circumstances for particle identification.

It is instructive to examine the variety of techniques available for estimating a single one of these parameters, the ionization loss, in emulsion tracks. From the photomicrographs in FIGURE 3 it will be

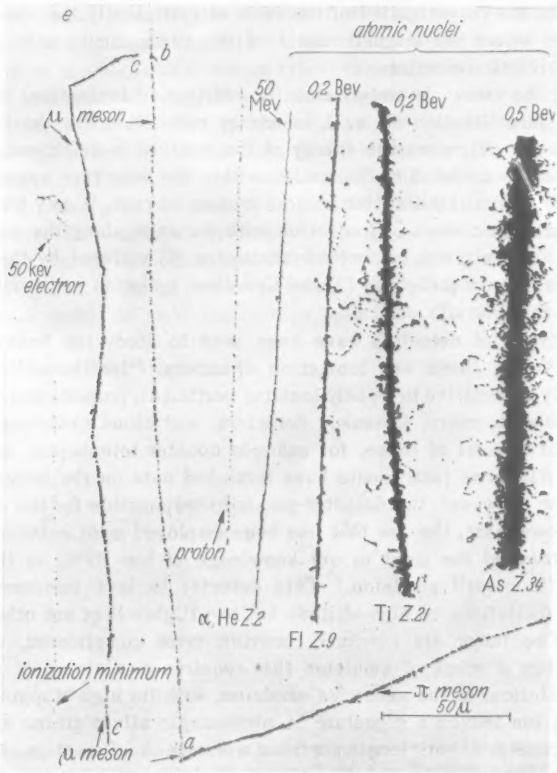

FIGURE 3. Photomicrographs of various types of tracks observed in a modern nuclear emulsion that has been exposed to cosmic radiation in a balloon flight (similar to that illustrated in FIGURE 1). Tracks of several heavy nuclei are shown at the right, energies being expressed in Bev./nucleon. The rate of ionization loss of the particle that produced the densest track is thousands of times that of the electron that left the track at the lower left. Delta rays can be seen along the tracks of the heavy nuclei (courtesy L. Leprince-Ringuet).

evident that the simplest method of ionization measurement—that of counting silver grains along the track—is of little help for the denser tracks in the more sensitive modern emulsion. In these tracks, single grains have generally coalesced into blobs, in which it is exceedingly difficult to resolve separate grains. By deliberately underdeveloping the emulsions (Bradt and Peters, 1950b) or by using intrinsically insensitive ones, the track of a densely ionizing particle can be thinned so that it is "grainy," that is, countable. Furthermore, by interleaving a stack with some insensitive layers, the thin tracks can be used in *addition* to, rather than *instead* of, the black tracks left by these particles in highly sensitive emulsions. In fact, a variety of methods has been developed for the latter case. First among these is the delta-ray density, that is, the frequency of secondary tracks of electrons ejected along the path of the particle, with energies exceeding, for example, 10 kev. Along the track of a relativistic alpha particle there are about 40 of these per cm. of track in emulsion and, for a fast aluminium nucleus, there are approximately 1600 of these per cm., producing a veritable fuzz of delta rays (FIGURE 3).

Because of the Z^2 dependence of the delta-ray density this method is rather sensitive, and it has been widely used, particularly since it is applicable over a wide range of Z values. Its principal drawback is the degree of subjectivity involved in judging whether a given delta ray has a range that meets the minimum energy prescribed. This is made difficult by the tortuous character of the low-energy electrons, and their obscuration, under some conditions, by the dense track of the parent particle. These difficulties set limits to the precision obtainable with this method. Nevertheless, with careful controls and cross-checks, the delta-ray density has been an exceedingly useful tool in particle recognition and identification. In investigations that require the highest precision, however, it should be replaced or supplemented by other methods.

Even dense tracks have gaps that have proved to be an excellent "handle" for estimation of ionization loss. The mean gap length, or the mean length of gaps exceeding some lower limit in size, or the integrated length of gap per unit length of track, as well as certain variants of these parameters have been applied successfully. In addition, the number of gaps per unit length, or the number exceeding some lower limit in size is sometimes useful. Recently it was found that the frequency of occurrence of single grains in dense tracks (which consist principally of blobs) is a rapidly determined and useful index of ionization (B. J. O'Brien, 1958).

Photometric devices capable of determining the effective opacity of a segment of track provide another method of ionization. A related technique that commonly yields more information but is more laborious is the "profile method," in which the projected width of a track is measured

at closely spaced intervals* (G. Alvial *et al.*, 1956). In practice, the first three methods – delta rays, grain counts, and gaps – have been most extensively used for ionization measurement.

As we have seen, ionization does not suffice, in general, for particle identification; therefore, the measurement of some additional physical characteristic is required. Thus, the combination of multiple coulomb scattering with ionization (S-I method) has provided a powerful tool for identification (Dainton *et al.*, 1951). Likewise, for emulsion stacks of sufficient volume to arrest by ionization loss a reasonable number of the lower energy heavies, the measurement of ionization *and* residual range make possible the determination of charge (Bradt and Peters, 1948; Freier *et al.*, 1948b).

A special phenomenon that depends on ionization and range is the tapering off of track width near the end of the range. For a heavy nucleus slowing down by ionization, there is at first a thickening of the track due to increasing ionization. Upon further slowing down, the density reaches a maximum that is followed by a progressive reduction in effective charge due to electron capture and a diminution in delta-ray production. These produce a terminal "thin-down" of the track's width (FIGURE 4). The residual range from the position of this maximum thickness is called the "thin-down length," and it has a characteristic value for each element (Freier *et al.*, 1948b).

Although, only a few years ago, the identification of heavy nuclei was a subject of controversy, the variety of methods now available for calibration makes it possible to assign a charge with considerable confidence. The assignment can frequently be verified by observing in the same stack a number of clear-cut examples of the break-up of a heavy nucleus into identifiable fragments (FIGURE 5). If a balance of charge between the incoming nucleus and the outgoing fragments can be established, this provides a test of one's calibration. Thus, for example, if a carbon nucleus collides with hydrogen, the tracks of three alpha particles and a proton might be observed to emerge. Given a sufficient length of track, it is not unusual to determine the charge to within one unit, at least for the lighter nuclei. By and large, a fairly clean separation of the elements is possible in a well-developed stack.

It has become customary to classify the various HPN into the following groups: helium (which may include He^3, as well as alpha particles); light nuclei (L component), consisting of Li, Be, and B; medium (M component), which includes C, N, O, and F; and heavy (Q component),† those with $Z \geq 10$. Of primary nuclei with $Z > 2$, the M group is the most abundant. Because the absolute fluxes (especially those extrapolated

*This method was devised primarily for identification of relatively slow particles, for example, hyperfragments, when only a short segment of residual range is available.

†We suggest Q as a designation for the heavy group, rather than H, which has been widely used, since the latter is the symbol for hydrogen, itself a constituent of the primary radiation.

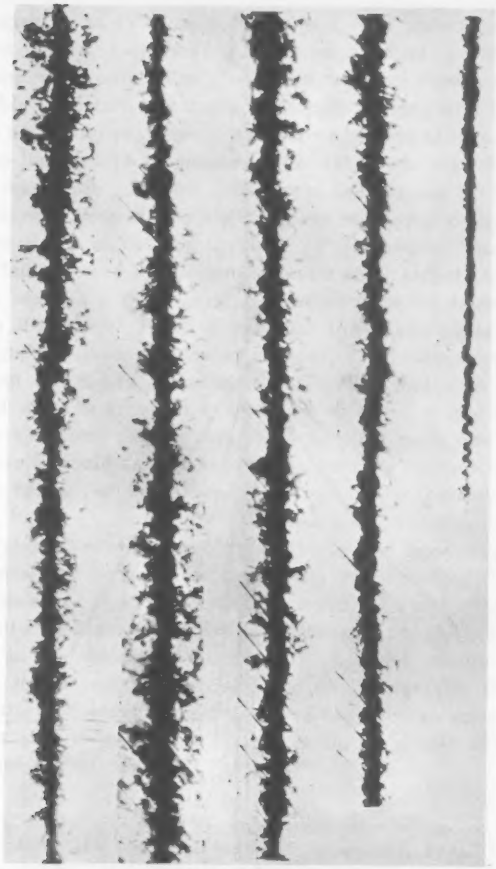

FIGURE 4. Photomosaic showing successive portions of the track of a heavy nucleus of the cosmic radiation that came to rest after producing a characteristic taper. The charge was estimated to be (42 ± 4)e from the thin-down length (the spiral appearance near the end of the track is due to a spurious effect, the buckling of the silver column when the emulsion contracts upon drying).

to the top of the atmosphere) of the various components are less reliably determined than the *relative* abundances, some workers have reported the values of certain ratios of these fluxes. We shall designate the ratio L to M fluxes by $R(L/M)$, and that of Q to M by $R(Q/M)$. We shall use R_o for the calculated ratios at the top of the atmosphere.

The correct identification of the heavy nuclei observed in a balloon flight, at a given level in the atmosphere, is only a part of the task of

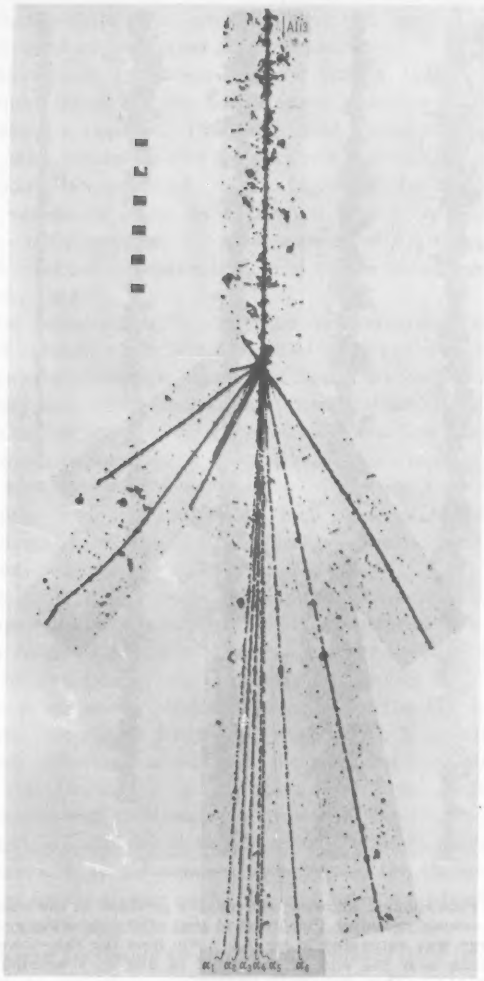

FIGURE 5. Upon collision with an atom of a photographic emulsion, a cosmic ray nucleus of aluminium breaks up into a jet of helium nuclei (α_1 to α_6). In addition, relatively slow fragments of the target nucleus leave other dense tracks. Scale: 1 div. = 10 μ (courtesy C. F. Powell).

deducing the character of the primary nuclei. As we have seen, the latter are subject to nuclear collisions with the air and packing material above the emulsion. Moreover, in view of the paucity of data, one cannot consider only vertical tracks, but must often accept particles arriving at

zenith angles up to 45 or even 60 degrees. The corresponding paths in air are, of course, longer. Thus, the heavy nuclei reaching the stack, even at depths of only 10 or 15 gm./cm.^{2} in the atmosphere, include a considerable admixture of secondary fragments along with the true primaries. It is necessary to correct for the contributions of these secondaries, as well as for the loss of primaries. To make such corrections, we must know the mean free paths for collision of the various HPN. In addition, we must know in some detail what happens in various collisions to the Q nuclei, the M nuclei, and the L nuclei. For example, how often does a heavy nucleus survive a collision without a sharp reduction in its charge, so that it remains a Q nucleus? How often does its fragmentation give rise to an M nucleus? To an L nucleus? How many alpha particles, on the average, emerge in the fragmentation jet? Similar questions may be asked about the M and L components. Suppose that it is common for a nucleus of a given group to generate in collision a fragment that still belongs to the same group. Then the absorption mean free path for that group of nuclei could be considerably longer than its collision mean free path. This has in fact been found to be true of the Q component (Danielson *et al.*, 1956).

By studying many collisions, considerable data have been accumulated on the mean free paths in emulsion and the so-called "fragmentation probabilities"* of the various groups of HPN. However, in order to use these data for extrapolation of particle intensities to the top of the atmosphere, it is necessary to convert the observed results on collision processes in emulsion to collisions in air. Altogether, the procedures for calculating primary fluxes from those observed at some depth in the atmosphere involve corrections of uncertain magnitude that have given rise to considerable controversy.

Primary Helium

The flux of primary helium is probably better known than that of either hydrogen or the heavier nuclei. Good measurements are available for at least three geomagnetic latitudes $\lambda = 55°$ N, 41° N, and in the vicinity of the geomagnetic equator. At 55° N excellent agreement was obtained between L. Davis *et al.* (1956) and C. J. Waddington (1954). Their values of the primary helium flux, obtained by quite different methods, and uncorrected for the contribution of secondary helium from the fragmentation of heavier nuclei, agree exactly, that is, 320 particles/m.^{2}/sec./steradian (it is useful to define a unit of cosmic ray intensity in such a way that the number of particles/m.^{2} sec. ster. may be called "peters"). At 41° N, so many independent measurements have been made of the helium flux that this number is probably the most precisely determined primary cosmic ray intensity. A summary of this work by W. R. Webber (1956), who has

*These are further discussed in a later section (TABLE 2).

himself contributed significantly to the measurement of intensities with Cerenkov detectors, reports a weighted average of 90 ± 3 peters at 41° N. This result, however, was uncorrected for the breakup of heavier nuclei into alpha particles. When the latter effect is taken into account, we arrive at a value of 82 ± 6 peters at this latitude.

Until little more than a year ago, the helium flux near the geomagnetic equator was not well known.* Then a value was obtained (Shapiro *et al.*, 1956) at $\lambda \simeq 10°$ N†; subsequently F. D. McDonald (1957) and W. R. Webber (1957) measured the helium flux at $\lambda \simeq 3°$ N, near Guam. The respective intensities reported by these observers are 22.3 ± 2 peters, 18.0 ± 1.9 peters, and 13.5 ± 1.7 peters. Although the two latter figures are not in close agreement, their weighted mean is close to the value expected from the flux observed at 10° N, since the magnetic cut-off energy is higher at Guam (3° N) than at the Galapagos Islands (10° N).

Using the data shown in TABLE 1, it is possible to deduce an integral energy spectrum for primary helium. To facilitate comparison between

TABLE 1

INTENSITIES OF "PRIMARY" HELIUM*

Geomagnetic latitude	$W_{min.}$ *(Bev.)*	*I (peters)*†
10°	6.98	23.7 ± 2.2
41°	2.53	90 ± 3
55°	1.23	320 ± 23

*Uncorrected for contribution of secondary He from fragmentation of heavier primary nuclei above the detector. These numbers are used to deduce the power-law approximation to the energy spectrum, given in the text.

†Particles/m.^{2} sec. sterad.

various observers, most of whom did not correct for the presence of secondary helium at balloon altitudes, the fluxes shown in the table are uncorrected for this effect. The magnetic cut-off energies are shown in the second column. $W_{min.}$ is the total energy per nucleon (kinetic energy plus rest mass). $N_{>W}$ is the flux of helium nuclei with energy per nucleon exceeding W. The integral spectrum can be written in the form‡

$$N_{>W} = (350 \pm 50)\, W^{-(1.45 \pm 0.15)}$$

*The following values had been obtained: an upper limit of 14 ± 20 peters (S. F. Singer, 1950; also Singer, 1958); 49 ± 13 peters (Pomerantz, 1954); and an upper limit of 38 peters (McClure, 1954).

†Exposure of the emulsions near the Galapagos Islands had occurred in September 1953.

‡This power law fits the energy spectrum at (total) energies W > 1.3 Bev./nucleon.

In view of the remaining uncertainties in the proton component, it would be rash to give a definitive figure on the relative abundance of primary helium and hydrogen. In measuring primary hydrogen, for example, with counter telescopes, there are many sources of spurious counts, such as nuclear interactions, their associated showers, and knock-on electrons. Fast secondary particles, for example protons and mesons, create difficulties, and the albedo, consisting of upward moving particles, contributes to the spurious flux. The latter effect has been largely corrected for with Cerenkov counters, capable of directional discrimination. Other secondary particles can be recognized by the use of guard counters that detect showers, but interpretation of the counting rates remains somewhat ambiguous.* However, steady improvement in the corrections for these various effects has given better estimates of the primary proton intensity in recent years.

From measurements at magnetic latitudes 2° S and 10° N, respectively, S. N. Vernov and A. N. Charakhch'yan (1953) and G. W. McClure (1954) independently found that the equatorial flux is nearly one half that previously assumed. McClure assigned an upper limit of 145 peters to the proton flux at 10° N. Combining this upper limit with the helium flux obtained at the same time and place by Shapiro *et al.*, one can deduce the following *lower* limit:

$$R_{\circ}\left(\frac{\mathrm{He}}{\mathrm{H}+\mathrm{He}}\right) \geq \frac{22}{167} = 13 \text{ per cent}$$

From his work at Guam, McDonald (1957) finds an upper limit of 115 peters for primary hydrogen at 3° N. Combining this with his own value for the helium flux at the same time and latitude, one obtains a lower limit to the same ratio of 18/133 = 13 per cent, in agreement with the earlier result at 10° N. McDonald also assigns a lower limit 95 ± 12 peters to the proton flux. Thus, one can deduce an *upper* limit to the ratio $R_{\circ}\left(\frac{\mathrm{He}}{\mathrm{H}+\mathrm{He}}\right)$ of 18/113 = 16 per cent. Further confirmation for the lower limit given above comes from another latitude and other observers: taking 82 peters as the (corrected) He flux at 41° N and Webber's upper limit of 526 for primary hydrogen at that latitude, we find $\frac{82}{608} = 13.5$ per cent. Thus it appears that the relative abundance of He to that of all singly and doubly charged primaries can now be bracketed between 13 and 16 per cent.

*It should be added that the measurement of primary hydrogen intensities with emulsions would be a very difficult undertaking, involving uncertain corrections due to secondary singly charged particles.

Primaries Heavier than Helium

A considerable body of data has been accumulated on the fluxes of HPN with $Z \geq 3$, and fair agreement has been obtained (at least for $\lambda \geq 41°$ N) on the absolute intensities of the M and Q components. However, many discrepancies persist, and this is especially true for the L component, which will be discussed in the following section. Among the experimental difficulties responsible for this situation are the following: (1) the primary intensities are small, so that it is difficult to obtain sufficient data; (2) even at balloon altitudes of 100,000 feet, enough material intervenes between the primaries and the detector to alter the primary distribution; and (3) investigators differ as to the magnitude of the corrections required to extrapolate from observations at balloon altitudes to the top of the atmosphere.

"Fragmentation probabilities" have been widely used to denote the mean number of particles of a given type produced in a specified type of interaction. For example, P_{QL} means the average number of light nuclei that emerge from the collision of a heavy one and $P_{M\alpha}$ is the average number of helium nuclei produced in fragmentation of an M nucleus (strictly, the term "probability" is a misnomer here, since P-values can exceed unity). TABLE 2 lists values of the fragmentation probabilities obtained in several laboratories. The numbers were obtained by studying the collisions of L, M, and Q nuclei with *light* nuclei in emulsion, for example, those of C, N, and O. This selection of target nuclei, based

TABLE 2

VALUES OF "FRAGMENTATION PROBABILITIES"*

	Radjopadhye and Waddington (1958)	*Cester et al. (1957)*	*Koshiba et al. (1957)*	*Noon and Kaplon† (1955)*
P_{QQ}	0.29	0.34		0.25
P_{QM}	0.46	0.27	0.21	0.27
P_{QL}	0.21	0.14	0.29	0.48
$P_{Q\alpha}$	1.23		1.57	2.07
P_{MM}	0.17	0.19	0.13	0.13
P_{ML}	0.23	0.23	0.17	0.42
$P_{M\alpha}$	1.27		0.87	1.42
P_{LL}	0.12	0.15	0.06	
$P_{L\alpha}$	0.79		0.47	

*For interactions with *light* nuclei in emulsion. These values approximate those for collisions with air nuclei. The symbols, for example, P_{ML}, are defined in the text.

†This column differs from the others; the numbers were *calculated* for air from observations in emulsion.

on the appearance of the fragmentation stars, provides data that can be reasonably applied to collisions with air nuclei. The last column of TABLE 2 tabulates fragmentation probabilities for air calculated by Noon *et al.* from data obtained in emulsion. Of the parameters listed in the table, two have a particularly important bearing on the extrapolation of the flux of light nuclei to the top of the atmosphere. These are P_{ML} and P_{QL}. It will be noted that the values of Noon and Kaplon are considerably larger than those of other workers; the former would therefore lead to *lower* estimates of the primary L flux. It is interesting to note that the earliest value of P_{ML}, calculated for air by Bradt and Peters (1950b), was 0.23, which is in agreement with the values subsequently obtained by most other observers.

From many balloon flights* at $\lambda \simeq 41°$ N, and several at $\sim 55°$ N and 3° to 10° N, it is possible to deduce extrapolated mean primary intensities for the medium and heavy nuclei at the top of the atmosphere; see TABLE 3.† Included with the balloon data are some results obtained from emulsions sent aloft in rockets (H. Yagoda, 1956). In principle, experiments in vehicles which soar above the atmosphere can provide the most unambiguous primary flux determinations. In practice, rocket flights have thus far been limited to durations of several minutes, and have therefore yielded data of low statistical weight.

The relative abundances of primary H, He, and heavier nuclei can be estimated, mainly from data at $\lambda = 41°$ N, as follows:

Hydrogen $\lesssim$ 85 per cent

Helium $\sim$ 14 per cent

$Z \geq 3$ $\gtrsim$ 1.4 per cent

Abundance of Li, Be, and B Primaries

The most controversial question concerning the heavy nuclei is that of the presence or absence of the elements Li, Be, and B in the primary cosmic ray beam. Since the typical abundance of these elements in the universe is believed to be exceedingly low, that is, 10^{-10} to 10^{-9} that of hydrogen, the incidence of a finite flux of these elements in the form of cosmic radiation would have important astrophysical implications. These light cosmic ray nuclei would most probably originate in the fragmentation of heavier nuclei by collision with the tenuous matter of interstellar space. The primary flux (if any) of L nuclei, as compared

*See references at the end of this article.

†It should be stressed that the absolute flux values cited in this paper, particularly those at $\lambda > 40°$, refer to the average "quiescent" cosmic radiation; that is, they are based on measurements made at times other than those of intense solar activity. During the past year evidence has been accumulating for a sharp decline in the latitude-sensitive primary intensities during the maximum of the 11-year solar cycle. In fact, a decrease to approximately one half of the quiescent intensity has been observed for both protons and HPN at higher latitudes (J. R. Winckler, 1957; P. S. Freier *et al.*, 1958).

TABLE 3

PRIMARY INTENSITIES* OF COSMIC RAY NUCLEI HAVING $Z \geq 6$

Magnetic latitude	*M nuclei* $6 \leq Z \leq 9$	*Q nuclei* $Z \geq 10$	$M + Q$
55°	11.5 ± 1.6†	3.8 ± 1.0	15.3 ± 1.9
41°	6.1 ± 0.7	2.5 ± 0.3	8.6 ± 0.8
$\lesssim 10°$	1 - 2	0.2 - 0.5	1.2 - 2.5

*Fluxes are given in peters (particles/m.^{2} sec. sterad.). The separate values for M and Q nuclei are based on eight investigations at $\lambda = 41°$. At the other latitudes they are each based on only two or three measurements (see references at end of this paper).

†The uncertainties are estimates that I have assigned. They are based on somewhat arbitrary weighting procedures.

with those of the heavier M and Q nuclei, would provide an index of the distance traversed by the latter and therefore of the age of these components of the cosmic radiation. It might also provide clues as to possible sites of origin of the radiation.

In 1950 Bradt and Peters measured the fluxes of heavy primaries at $\lambda = 30°$ N and, extrapolating their results to the top of the atmosphere, found that the ratio $R_o(L/M)$ is less than 0.1. They concluded that Li, Be, and B are "almost or entirely absent" in the primary cosmic ray beam. Later this question was investigated by many other workers, among them the cosmic ray groups at the Universities of Bristol, Rochester, Chicago, Turin, Sydney, Iowa, and Göttingen. For this ratio of light to medium elements, the investigators obtained values varying from 0.3 to 1.2. Considering the results of all these investigations, one can derive a mean value for R_o (L/M) of 0.4 to 0.65, depending on the relative weights assigned to the various measurements, and on the choice of values of P_{QL}, P_{ML}. Thus, in their discussion of the presence of D, Li, Be, and B in our galaxy, Burbidge *et al.* (1957) felt it safe to write that "in the primary cosmic radiation the abundances of Li, Be, and B are comparable with those of C, N, and O."

If one selects the three investigations having the greatest statistical weight, their combined result* is R_o $(L/M) = 0.34$. From an extensive study of HPN fragmentations, V. Y. Rajopadhye and C. J. Waddington (1958) concluded: "The observation of a ratio of $L/M > 0.25$ at depths 10-15 gm./cm.^{2} is incompatible with the assumption that there are no L nuclei at the top."

Early this year, however, the group at the Tata Institute in Bombay, India (M. V. K. Apparao *et al.*, 1958) reported their new finding that R_o (L/M) is 0.075 ± 0.08. Their emulsions were exposed at an atmospheric depth of only ~ 6.6 gm./cm.^{2}, and their observations were made under

*If the result of Apparao *et al.* (discussed below) is combined with these three, the mean value is 0.28.

total thicknesses of matter as low as 8.5 gm./cm.^{2}. Therefore they considered it safe to extrapolate to the top of the atmosphere, using their own observations and those of certain other groups with adequate statistics. They plotted the L/M ratios at various atmospheric depths against the amount of overlying material and avoided the use of "fragmentation probabilities" or mean free paths. Their conclusion, that the results are consistent with a negligible flux of L nuclei among the HPN at the top of the atmosphere, is thus at variance with those of most other observers.*

Some Remaining Problems

In our brief glimpse of HPN research, we have perforce omitted many significant developments, such as studies of the lowest energy He primaries, evidence for discrepancies between geomagnetic latitudes and cosmic ray latitudes, methods of estimating the very high energies of HPN nuclei above the latitude-sensitive region, evidence that the different HPN have similar velocity spectra, and various time variations of the HPN. We should at least mention a few of the unsettled problems that remain to be explored:

Chemical and isotopic composition of the primaries. Is there a significant flux of Li, Be, and B? Is deuterium present among the primaries? What is the ratio He^3/He^4? Are odd-Z nuclides less abundant than those of even Z? Is there an appreciable flux of heavy elements well beyond Fe (for example, $Z > 40$)?

Energy spectra of HPN. Further flux determinations near the geomagnetic equator are required. Additional energy measurements up to the highest energies, using HPN fragmentations, would permit comparisons with the proton spectrum. The possibility of extragalactic origin for a portion of the cosmic radiation may hinge partly on the question: are the largest air showers (of total energy $\sim 10^{18}$ or 10^{19} e.v.) initiated only by HPN of rather high Z, or by protons as well? In the former case, the energy per nucleon might be two orders of magnitude smaller and perhaps consistent with a galactic origin. The extreme rarity of these events makes the experimental problem difficult.

Time variations. The existence of a pronounced day-night effect is still an open question. What happens to the HPN flux and spectrum during the time of a giant solar flare? Does the drop in primary intensities at the height of the eleven-year solar cycle – a large effect at the higher latitudes – also occur near the magnetic equator?

Prospects for Direct Exploration of the Primary Radiation

The recent availability of thin plastic balloons capable of reaching pressure altitudes < 3 gm./cm.^{2} permits exposures so close to the top

*This conclusion is not inconsistent with that of J. Linsley (1956), who found an upper limit for $R_0(L/M)$ of 0.25.

of the atmosphere that corrections for fragmentation of HPN above the emulsions become quite small. Observations at these altitudes should yield conclusive information regarding the presence and abundance of Li, Be, and B among the primaries.

In addition, powerful rockets have been developed that could propel a package of cosmic ray instruments up to altitudes of thousands of miles. With suitable arrangements for safe re-entry into the atmosphere and subsequent recovery, it will become possible to send a sizable stack of nuclear emulsions out to distances of one or more earth radii. In fact, a group of cosmic ray physicists is planning this type of experiment,* and a feasibility study showing how such an exposure and recovery might be accomplished has been made (H. Sohn, 1957). Among the many incentives for exploring the regions far outside the earth is the fact that the terrestrial magnetic field intensity falls off rapidly with distance.

Ultimately, it will become possible to recover apparatus from satellites after an exposure of preselected duration in orbit. This will permit the collection of data on exceedingly infrequent phenomena. For example, rare nuclei of very high atomic number, or evidence for the presence of antimatter, might be recorded in this way. Of course, valuable data on the extraterrestrial cosmic radiation and related phenomena can be obtained with suitable detectors by telemetry; this is already being demonstrated.

The cosmic ray observations from the two Explorer satellites, 1958 α and 1958 γ, now circling the earth, are being made with a simple apparatus consisting essentially of a single Geiger tube, with an associated scaling circuit and telemetry system. Preliminary analysis of these data has already yielded a startling result (J. A. Van Allen *et al.*, 1958): at altitudes exceeding 1000 km. the Geiger tube is apparently blanked by an intense radiation field. The authors surmise that this radiation is closely related to the soft radiation previously detected during rocket flights in the auroral zone. If their estimate of the intensity is correct, then a man bathed in this sea of radiation would receive a week's "permissible dose" within 2 hours.

As further cosmic ray experiments are carried out in these hitherto unexplored regions, we may expect other surprises.†

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*Committee for Extra-Atmospheric Cosmic-Ray Research of the National Academy of Sciences, Washington, D. C., under the chairmanship of G. Groetzinger.

†A cosmic ray experiment involving an electron-collecting ionization chamber, which will register the ionization loss of particles traversing it, is planned for the near future (G. Groetzinger, M. Pomerantz and P. Schwed, private communication).

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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF BIOLOGY

THE GIBBERELLINS: POWERFUL PLANT GROWTH REGULATORS*

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The beginning of the gibberellin story is a familiar one to many people. These remarkable substances, gibberellin *A*, gibberellin *B*, and gibberellic acid, are the metabolic products of a fungus, *Gibberella fujikuroi*, which causes the so-called "foolish seedling" disease of rice, not uncommon in Japan. A characteristic early symptom of the disease is the elongation of the rice shoot, so that in an infected crop diseased plants are much taller than healthy ones. In later stages of the disease, however, the fungal invasion causes necrosis of the basal tissues of the stem and the seedlings become weak or die. A Japanese worker, Kurosawa, showed in 1926[1] that symptoms of overgrowth could be induced in rice seedlings by applying a cell-free filtrate from liquid cultures of *G. fujikuroi* to the roots. Two biologically active materials, gibberellins *A* and *B*, were isolated in pure form by Yabuta and Sumiki in 1938.[1] Gibberellic acid, isolated by Curtis and Cross[2] in England, is a tetracyclic dihydroxylactonic acid, identical to gibberellin *X* reported by Stodola *et al.*[3] in the United States, and distinct from gibberellin *A*.

In spite of the interest in plant growth regulators, these gibberellins claimed little attention until 1954, when Brian and his co-workers in England published an article on their growth-promoting properties.[4] Since that time, many reports have enumerated the effects of gibberellins on all parts of various plants in different physiological stages, from germination of the seed to flowering and fruiting. Stowe and Yamaki[1] have given a review of this work (202 references) up to 1957. Many other accounts published during 1957 and 1958 have described responses of plants to the application of gibberellins.

No attempt will be made here to cite all of the literature on the subject. Rather, a brief general review of some of the physiological and morphologic effects of the gibberellins will be offered, together with examples of specific experimental results obtained at the Boyce Thompson Institute for Plant Research during 1956 and 1957.†

Seed Treatment

Seed may be treated by soaking in solutions of gibberellic acid or by treating with dust before planting in soil. In greenhouse tests, the germi-

*This paper, illustrated with slides, was presented at a meeting of the Section on May 12, 1958.

†These experiments were supported in part by Eli Lilly and Co., Indianapolis, Ind.

nation of Perfection peas, lima beans, and White Dutch clover seeds was hastened by treatment with gibberellic acid, and the heights of the seedlings of the first two were increased. The speed of germination was also increased in corn, barley, and wheat, but not in oats as a result of the treatment. Of the grains, corn was the only one that showed indications of increased height from gibberellic acid treatment, and this effect was from soaking treatment rather than from dust application.

Duplicate lots of 100 seeds were treated, except for barley and clover, of which duplicate lots of 200 were used. For dust treatment, each lot was placed in a 2-oz. bottle with 500 mg. of talc or 500 mg. of talc containing gibberellic acid in concentrations of 10, 20, 40, or 80 mg./10 gm. of talc. The bottles were placed on a mechanical roller until the seeds were well coated with the dust. Wet treatments were in tap water or in tap water containing 2.5, 5.0, or 10.0 p.p.m. of gibberellic acid. The amount of solution placed on each seed lot was predetermined as the amount that would be completely absorbed by the given lot of seeds in 24 hours at room temperature.

The most striking results were obtained with treated lima bean and pea seeds. This is illustrated for lima beans in FIGURE 1. The extended growth after treatment with gibberellic acid is especially evident in the long first internodes. Theoretically, this is desirable in the field, for

FIGURE 1. Lima bean seedlings from seeds dusted and planted in soil in the greenhouse. Left to right: seeds dusted with talc or with talc containing 10, 20, or 40 mg. gibberellic acid + 10 gm. of talc.

it would permit mechanical harvesting of beans that must be hand-picked at present. Actually, however, severe lodging of bean plants in a field at Yonkers, N. Y. during the summer of 1957 indicated that any increase in stem height produced by gibberellic treatment of the seed prior to planting would be of no advantage at the time of harvest. These field tests were conducted with White Navy pea beans, red kidney bush beans, and Clark's bush lima beans. All seeds were planted in Latin Square arrangements after no treatment, or treatment with talc alone, or in combination with gibberellic acid at rates of 1 or 8 mg./gm. of talc. There was some indication of earlier flowering and maturity as a result of gibberellic acid seed treatment, but further tests are needed to establish this effect.

The stimulating effect on growth of Perfection peas after seed treatment is shown in FIGURE 2. Here it is also seen that gibberellic acid does not protect the seedlings from damping off, but it can be combined successfully with captan that controls damping off organisms.

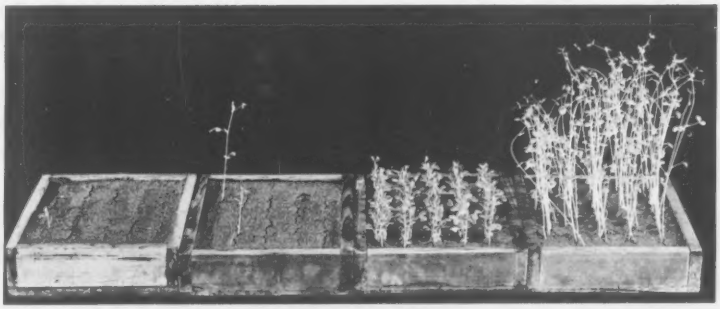

FIGURE 2. Seedlings of Perfection peas from seeds treated with dust before planting in *Pythium*-infested soil. Left to right: talc; talc + gibberellic acid; captan; captan + gibberellic acid.

Among the published reports on the effect of seed treatment with gibberellins is that of Wittwer and Bukovac,[5] who reported early emergence and rapid growth of peas and beans following treatment.

There seems to be some difference of opinion regarding the value of gibberellins for cotton seed treatment. Some of the potential favorable effects, such as increased and early emergence, increased boll set, higher yield, and longer fiber, have been pointed out.[6] Ergle and Bird,[7] however, found that gibberellic acid seed treatment reduced the cotton stand from approximately 60 to 14 per cent in fine loam, following rain and compaction of the soil after planting. The injury resulted from the breaking of the hypocotyls before the cotyledons were pulled out of the soil.

In general, seed treatment seems to be of doubtful value, since much depends upon the type of soil and the climatic conditions following

planting. Spraying the plants at some stage after emergence appears to produce more predictable results.

Effect on the Growth of Grass

In the fall of 1956, several plants still growing in the field at Yonkers, N. Y., were sprayed with gibberellic acid. Among these was crab grass that had started to take on autumn coloration. Spraying with gibberellic acid caused this grass to become green again and to grow rapidly. Subsequently, a series of experiments with Kentucky bluegrass were performed in Indiana. The results showed that the fall growth of bluegrass was significantly increased by spraying with gibberellic acid at the rate of 2 or 4 oz. per acre. Fresh and dry weights of the plants were increased, especially when fertilizer was added.[8] Observational plots of Kentucky bluegrass were sprayed in Yonkers, N. Y. late in November 1956. Sprayed plots were much greener throughout the winter than control plots. FIGURE 3 shows some of these plots as they appeared on March 2, 1957 after a light snow. The control portions were completely covered with the snow, while the grass sprayed with gibberellic acid had grown sufficiently to extend above it.

The idea of using gibberellic acid to increase the growth of pastures early in the spring and late in the fall—in short, to extend the growing season of grasses—has been pursued rather extensively. Many of the results have not been published, but a recent report of comprehensive

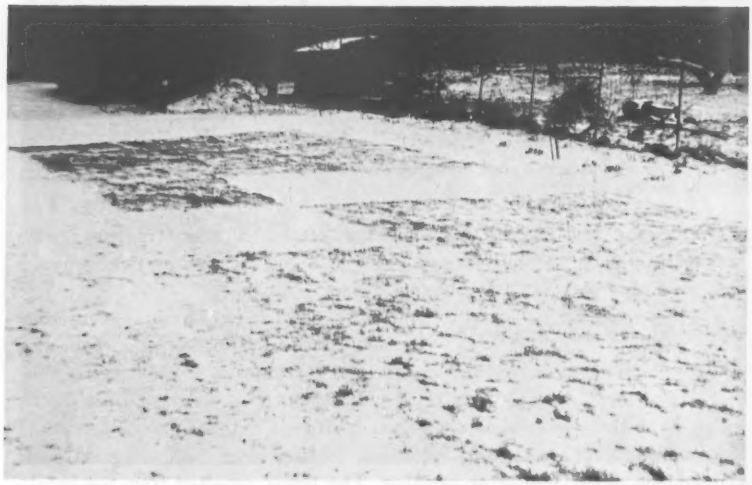

FIGURE 3. Grass plots photographed March 2, 1957 at Yonkers, N. Y. Control plots covered with snow; plots sprayed with gibberellic acid the previous November, with growth extending above the snow.

tests has come from England.[9] It was found that the growth of all components of grassland was stimulated by gibberellic acid sprayed at the rate of 2 oz. in 100 gal. of water per acre, as shown by the first cutting after treatment. The second cutting showed a corresponding reduction in growth; the effect of the gibberellic acid had completely disappeared at the third cutting. It was concluded as a result of these experiments that gibberellic acid has no advantage over fertilizer for increasing grass growth except in the spring and autumn, when fertilizer action is slow.

Robbins[10] found that adult plants of *Hedera* reverted to the juvenile form upon treatment with gibberellic acid. In this case an actual change of form accompanied growth stimulation. Alteration of leaf size and shape in tomato, pepper, tobacco, and African violet follows spraying with gibberellic acid.[11] The leaves of tomato plants develop smooth edges instead of the normal indented edges. The same formative effect was obtained at Yonkers by dipping the roots of young tomato transplants in talc containing gibberellic acid (FIGURE 4).

Effect on Flowering and Fruit Set

With the stem elongation effect of gibberellins established, attention naturally turned to the effect on flowering. Many studies have been reported and even more have been made on this phase of gibberellin responses. A striking response is the induction of flowering in biennials in one year instead of the two normally required. One of the first of these reports was that of Lang,[12] who described flowering of *Hyoscyamus niger*. Treatment of biennials was later expanded to include cabbage, kale, collard, rutabaga, turnip, and carrot.[13] The flowering of foxglove, English daisy, stock, and pansy was also hastened by treatment with gibberellin.[14] Long-day annuals have been made to flower under short photoperiods after such treatment.[15] More recently[16] an increase in the flowering response of *Xanthium*, a short-day plant, has been reported. An attempt to force the flowering of poinsettia with gibberellic acid under long-day conditions in the greenhouse at Yonkers failed, although stem growth was increased by the treatment (FIGURE 5). The size of the flowers also can be increased in such plants as hydrangea and geranium.[20]

Growth, flowering, and fruit set of tomatoes have been stimulated by gibberellin,[17] which also causes the set of parthenocarpic fruits[18] and breaks "summer-induced" dormancy of the tomato.[19]

The set and size of fruit on Black Corinth and Thompson seedless grapes have been increased significantly by spraying the young flowers with gibberellin.[21] Furthermore, increased length of the pedicels as a result of the treatment produced loose bunches that were not so susceptible to fungus injury. Two expensive hand operations, girdling and thinning, can thus be eliminated.

FIGURE 4. Tomato transplants in the greenhouse. Roots dipped in (a) talc or (b) talc containing gibberellic acid (80 mg. gibberellic acid + 10 gm. of talc) at the time of transplanting to flats. Note the effect on leaf shape.

Effect on Bud Dormancy

Potatoes. Single bud pieces of dormant and nondormant potatoes respond to soaking in gibberellic acid solutions by quicker emergence from the soil and more rapid early growth in the greenhouse. Varieties

FIGURE 5. Poinsettia plants after repeated treatments of the tips with aqueous solutions of gibberellic acid. Left to right: water control, 1, 10, 50, and 100 p.p.m. gibberellic acid.

tested at Yonkers have included Kennebec, Chippewa, Katahdin, Russet Burbank, and Green Mountain. Cylindrical, one-bud seed pieces (2 × 2 cm.) of the tuber were soaked, bud side down, in shallow solutions for periods of 30 sec. to 60 min., or were dipped into the solutions and immediately inverted to dry. Solutions used were combinations of gibberellic acid at 0, 0.1, 1.0, and 10.0 p.p.m., and α-napthaleneacetic acid at 0, 1.0, and 10.0 p.p.m. A dip in the solution was effective, and extended soaking for as long as 15 min. reduced the germination, especially in water control.

Some of the effects on Green Mountain variety 19 days after planting treated seed pieces in soil are shown in FIGURES 6 and 7. In each photograph, the flat on the left contains buds given a dip treatment, while the buds planted in the flat on the right were soaked 15 min. in the various solutions. The stimulation of growth by treatment with gibberellic acid, at 1 or 10 p.p.m. is evident (FIGURE 6). The addition of α-naphthaleneacetic acid at 1 p.p.m. to gibberellic acid at 1 p.p.m. increased the speed of emergence and contributed to uniformity of stand (FIGURE 7), while the addition of α-naphthaleneacetic acid to 10 p.p.m. gibberellic acid offered no advantage over the gibberellic acid alone. A stronger concentration of α-naphthaleneacetic acid (10 p.p.m.) was of no advantage in combination with gibberellic acid and prevented

FIGURE 6. Green Mountain potato plants 19 days after treating single buds with gibberellic acid at 0 p.p.m. (a), 1 p.p.m. (b), or 10 p.p.m. (c). Flat on the left in each photograph planted after dip treatment and flat on right after soaking in solutions for 15 min.

germination when used alone. The same germination and growth as with water control were achieved by the use of α-naphthaleneacetic acid alone at 1 p.p.m. The same general effect has been noted in a similar series of tests with the Russet Burbank variety. It appears that it may be advantageous to use α-naphthaleneacetic acid in combination with gibberellic acid to ensure prompt emergence of buds of potato tubers without abnormal, extended growth.

Plants of both Green Mountain and Russet Burbank varieties were grown further in flats in the greenhouse and were harvested 23 days after planting when tuber formation was noted. Sample lots are pictured in FIGURE 8 for the Russet Burbank variety. The increased number of tubers formed after treatment with gibberellic acid as compared with those in the water control is striking. Furthermore, gibberellic acid at 10 p.p.m. caused the production of larger tubers. None of these tubers exceeded 30 mm. in diameter and they had been grown in shallow soil,

but the results are suggestive. It should be pointed out that tuber formation after dip treatment in water was better than after 15 min. soaking in water, as pictured in FIGURE 8. However, all gibberellic acid treatments, with the exception of those combined with the higher concentration of α-naphthaleneacetic acid (10 p.p.m.), showed increased tuber pro-

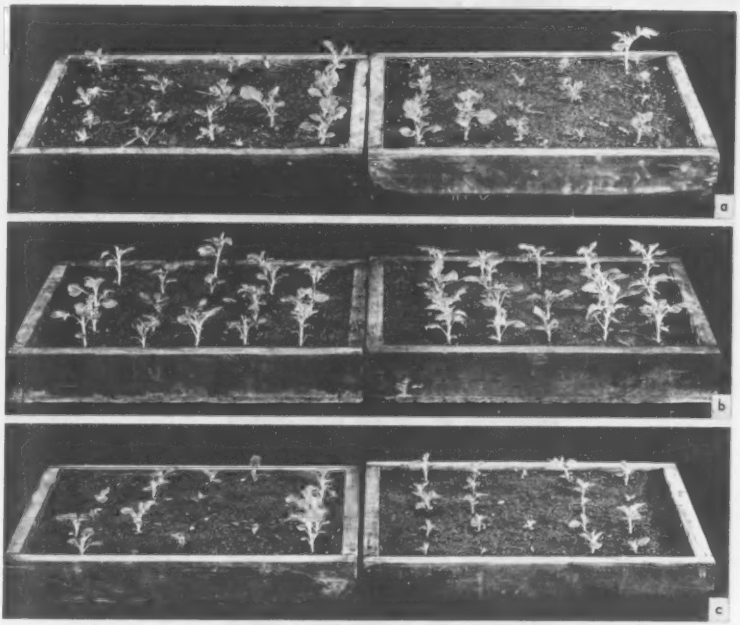

FIGURE 7. Green Mountain potato plants 19 days after planting single buds treated with 1 p.p.m. gibberellic acid in combination with α-naphthaleneacetic acid at 0 p.p.m. (a), 1 p.p.m. (b), or 10 p.p.m. (c). Flat on the left in each photograph planted after dip treatment and flat on right after soaking in solutions for 15 min.

duction over that of the water controls. Although the conditions for tuber production were far from ideal in these greenhouse experiments, the results are suggestive.

Rappaport *et al.*[22] have reported on a field test in which tubers of White Rose and Russet Burbank potatoes were dipped in solutions of gibberellin. Sprouting was stimulated, but concentrations as low as 10 p.p.m. were found to be detrimental to plant growth and subsequent tuber development. Lower concentrations (0.5 to 5.0 p.p.m.) shortened the rest period of the tubers with little injury. The same workers found that preharvest sprays of gibberellin produced tubers that, when cut

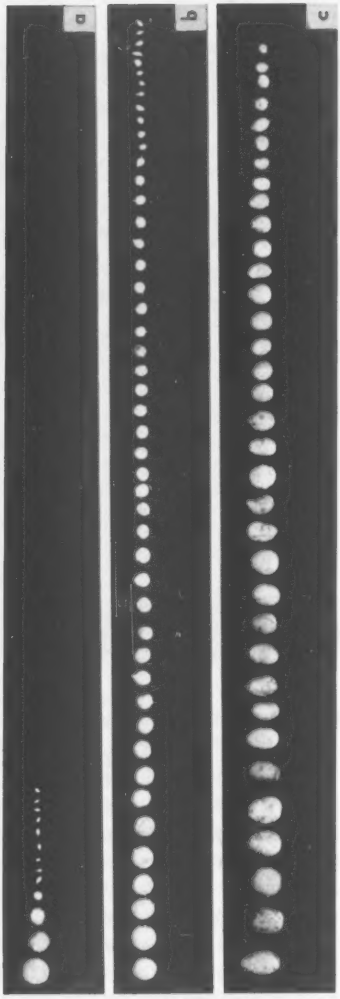

FIGURE 8. Tubers formed by 25 Russet Burbank potato plants produced from single-bud seed pieces soaked for 15 min. in water (a), or in gibberellic acid solutions at 1 p.p.m. (b), or 10 p.p.m. (c).

and planted as seed pieces, germinated rapidly. They conclude, however, that the commercial importance of these findings is still to be determined.

Trees. Donoho and Walker[23] have reported the replacement of part of the normal cold requirement of peach trees by gibberellic acid spray. Dormant, one-year-old grafts of Elberta peach brought into the greenhouse at Yonkers on September 28, 1956 and held there over winter are pictured in FIGURE 9b 20 days after a January treatment of each bud on the plant with water or with 1, 10, or 100 μg. of gibberellic acid. Growth started first after the highest dosage of gibberellic acid and the new stems were longest after that treatment. Spraying the entire plant with 1000 p.p.m. gibberellic acid also induced growth that was more general than that resulting from individual bud treatment, though the extension of the new growth was reduced. All of the old leaves were removed from the plants about a month before treatment.

Dormant buds of Jonathan apple grafts with old leaves still on the plants failed to respond to gibberellic acid treatment. However, when all the old leaves were removed before spraying with 1000 p.p.m. gibberellic acid new leaves were produced (FIGURE 9a). The presence of only 2 old terminal leaves on the apple plant reduced the effectiveness of the gibberellic acid spray.

Limited tests made in the winter of 1957-1958 confirmed the results with Jonathan apples. However, it was found that old leaves of the Elberta peach did not alter the gibberellic acid effect as much as did old leaves of the Jonathan apple. The implication that there is a competition between natural growth-regulating substances and the applied gibberellic acid is deserving of further study. Such a study is contemplated.

Varying effects of gibberellic acid on growth of different trees have been reported,[24-26] but in general are not as spectacular as effects on herbaceous forms.

Dwarf Plants

Brian and Hemming[27] reported that slow-growing varieties of peas will respond more than fast-growing varieties to applications of gibberellic acid. They also found a differential growth response to applications of gibberellic acid between tall and dwarf varieties of broad bean and French bean, suggesting that this response may be under genetic control. Phinney[28] investigated the effect of gibberellin on dwarf corn, and found that four dwarf mutants responded to treatment with gibberellic acid with extended, normal growth, while two other mutants failed to respond.

Extended growth of physiological dwarfs produced by planting excised embryos of nonafter-ripened seeds of crab apple results from the application of gibberellic acid, which substitutes in this case for low temperature treatment.[29]

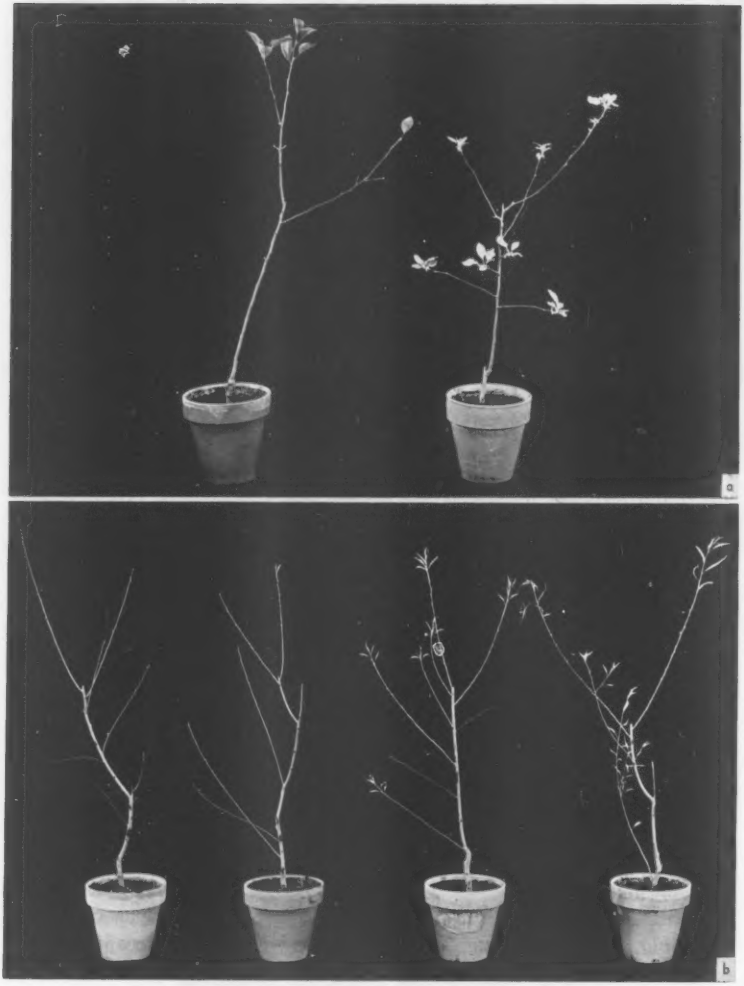

FIGURE 9. Effect of gibberellic acid on bud dormancy. (a) Jonathan apple plants 15 days after spraying with 1000 p.p.m. gibberellic acid; left: old leaves left on; right: old leaves removed. (b) Elberta peach plants 20 days after treatment of each bud with (left to right) water, or 1, 10, or 100 μg. gibberellic acid.

Virus-caused stunting in plants of China aster, hybrid sweet corn, and Crimson clover can also be reversed by the application of gibberellic acid.[30] Thus, it is seen that a dwarfish condition of many plants, whether genetic, physiological, or disease-produced, can be eliminated by gibberellic acid.

Tree Peony Epicotyl Dormancy

At ordinary temperatures seeds of the tree peony germinate readily to form roots. However, green shoots rarely appear above ground in the greenhouse, indicating that the portion of the seedling that forms the shoot is dormant. This condition has been called epicotyl dormancy, which can be broken by subjecting the seedlings, with the roots already growing, to a temperature of 1° to 10° C. for a period of 2 to 3 months. Gibberellic acid has been found to promote the growth of dormant epicotyls of the tree peony, replacing the need for low temperature pretreatment for the production of green shoots in the greenhouse.[31] Application of 1, 10, or 100 μg. to the hypocotyl of the germinated seed before planting in soil caused the emergence of green shoots above ground in 3 weeks or less. The most rapid devdlopment of the epicotyl followed treatment with 100 μg. gibberellic acid, but more normal growth resulted from the lower dosages. This is the only chemical that has been found to break the epicotyl dormancy of the tree peony.

Morphological studies have shown that differentiation of epicotyledonary tissues and elongation of the epicotyledonary shoot proceeds slowly in untreated seedlings in soil in the greenhouse.[31] After application of gibberellic acid, however, both differentiation and elongation were rapid, as shown by microscopic examination of sectioned material. Elongation of the epicotyl induced by gibberellic acid or by a period of 8 weeks at 5° C. was characteristic of seedlings that were capable of producing green shoots in soil in the greenhouse. Such elongation did not occur in untreated seedlings that failed to produce green shoots. Elongation of the petiole is due to an increase in cell size and cell number.

Natural Occurence of Gibberellinlike Substances

The gibberellins are produced by a specific fungus in the course of its normal metabolic activities. Do other fungi produce such stimulants? Curtis[32] has made a survey of approximately 1000 fungus and 500 actinomycete culture filtrates for effect on the growth of dwarf corn seedlings, but none of these caused any growth stimulation.

More success has been attained in the field of higher plants where Phinney and his co-workers in California[33] have found gibberellinlike compounds in parts of several different plants. One of the richest sources found is the immature seed (jelly stage) of the wild cucumber, *Echinocystis* which, according to West,[34] has an amount of gibberellinlike activity equivalent to about 1 gm. of gibberellic acid per 4 bushels of fruit. Endosperm material from *Echinocystis* seed caused bolting in biennials.[35]

Practical Considerations

Much has been written about the agricultural and horticultural possibilities of this remarkable growth promoter. Unfortunately, many of the early claims have not been substantiated by experimental results.

In addition to articles already cited above, recent ones by Nelson,[36] Merritt,[37] Pirone,[38] and Wittwer and Bukovac[39, 40] will give the reader some idea of these possibilities. The stimulation of pollen germination and pollen-tube growth[41] may make possible the removal of incompatibilities in some plants. The effect on bacteria and other lower organisms has been shown to vary, but thus far these studies have been rather limited.[42, 43]

From the point of view of the plant physiologist this latest of the plant growth-regulating chemicals offers a new approach to many problems that have remained unsolved. For example, the interrelations of temperature, light, auxin, and gibberellic acid may reveal new pathways for the elucidation of the phenomena of dormancy and the flowering of plants. Some progress has already been made along this line,[44-50] and no doubt other studies are being conducted. We are passing the initial stage of excitement following the discovery of gibberellins, and we are settling down to a real study of its mechanism of action.

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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF MATHEMATICS AND ENGINEERING

STUDIES OF PEAK BOILING*

By Thomas B. Drew

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This paper summarizes our present understanding of the mechanism of boiling water and, together with the illustrations, reports briefly some of the studies completed and under way in the heat transfer laboratory operated at Columbia University for the United States Atomic Energy Commission, Washington, D. C.

Boiling connotes continued production in a liquid of bubbles of its own vapor. Because vaporization is usually attended by a substantial increase in enthalpy, maintenance of active boiling generally requires a high rate of heat flow from external sources; however, when ginger ale, for example, "boils" in a glass on the table, such is not always the case.

Typically, the bubbles form on the heating surface, disengage themselves, and rise through the liquid. On the other hand, surfaces differ with respect to the degree of supersaturation needful for the formation of bubbles upon them; thus, if the heating surface is near an unheated wall that is a superior catalyst for bubble formation, bubbles may form there and not on the heating surface. Especially if the main body of the liquid is subcooled, bubbles formed on a heating surface may not disengage themselves; they sometimes appear to grow rapidly and just as rapidly to disappear completely without leaving their point of attachment. At high rates of boiling they sometimes assume irregular sacklike shapes, sacks with their mouths attached to the heating surface and their bottoms waving in the liquid. Quite commonly, if the liquid is nearly saturated, new bubbles repeatedly grow at specific points on the heating surface, but under subcooled liquid no such "memory" on the part of the surface exists. In principle, given sufficient supersaturation, bubbles can form in the body of a liquid, but in practice this is not commonly observed. These forms of boiling are collectively "nucleate boiling": vapor nuclei formed in the liquid or existing at its interfaces find locations suited to their growth in liquid of the ambient temperature. "Local nucleate boiling" serves to describe boiling in subcooled liquids in which bubbles exist only on or near the heating surface. FIGURE 1 is a microflash photograph (40 to 50 μ sec.) of local boiling on a vertical tube.

"Film boiling" occurs when a heating surface is sufficiently hotter than the boiling point to maintain a stably flowing layer of vapor between itself and the liquid. If the vapor has no other adequate path for escape, large bubbles are irregularly formed at the vapor-liquid interface as the

*This paper, illustrated with slides and motion pictures, was presented at a meeting of the Section on May 16, 1958.

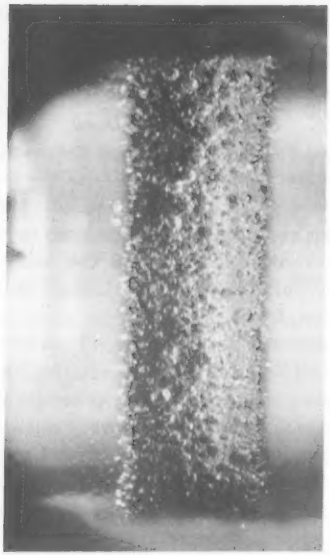

FIGURE 1. Local nucleate boiling on one-half in. OD nickel tube. Water at 92.6 pounds/square in. abs. subcooled 175° F., flowing 0.130 feet/sec. $\frac{Q}{A\theta}$=600,000 (B.T.U. per square foot) per hour. Wall temperature 370° F.

newly formed vapor releases itself. Film boiling can occur only at large temperature differences: the heat for evaporation must flow by radiation and convection through a layer of gas in order to reach the surface of vaporization. In contradistinction, the chief path of heat flow during nucleate boiling lies from the heating surface through the liquid phase to the bubbles' interfaces or into the body of the liquid. Most of the work discussed herein concerns nucleate boiling and mixed regimes. The surface temperature needful for film boiling of water is above the melting point of many metals interesting as heating surfaces in nuclear reactors.

FIGURE 2 shows the typical variation of local heat flux density with heating surface temperature when water is boiled. The actual location of the curve varies with the pressure and temperature of the liquid. The slope of the steeply rising line at the left varies somewhat with water velocity and degree of subcooling. The level of the peak at the left is usually raised by increase of pressure and subcooling. This is shown by FIGURE 3. The heat fluxes at the peak are truly huge [1 to 3 million (B.T.U. per square foot) per hour] even though the velocity of the liquid is relatively low. When heat is supplied electrically or by a nuclear reaction the heating surface typically melts (burnout) if an attempt is made to

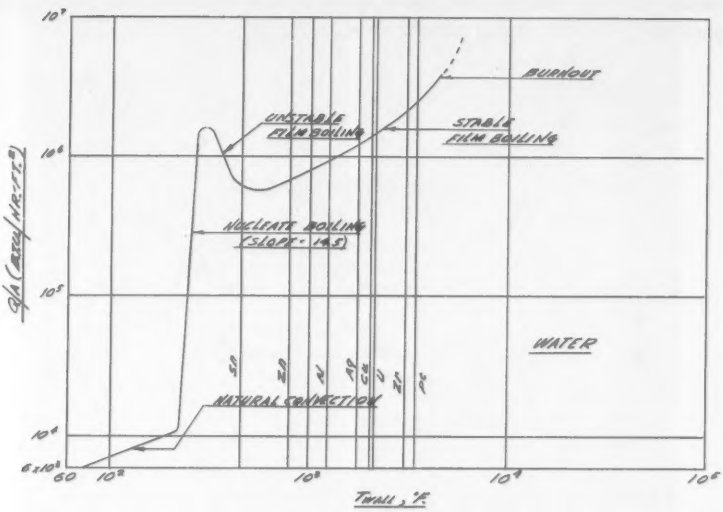

FIGURE 2. Typical boiling curve for water.

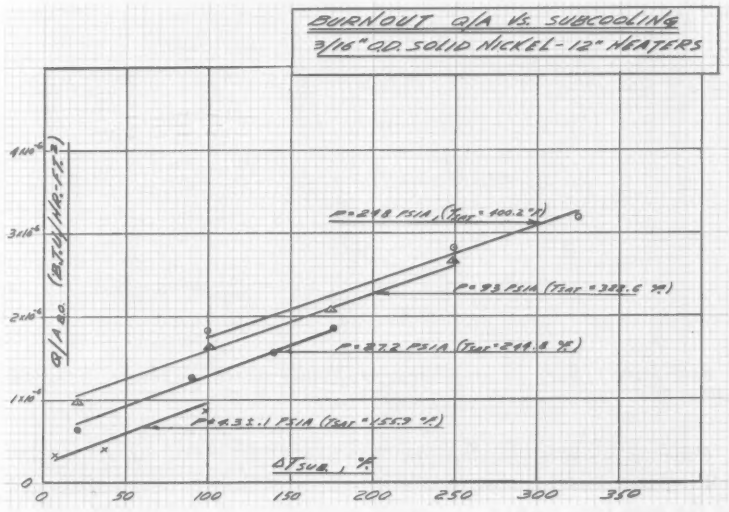
BURNOUT Q/A VS. SUBCOOLING
3/16" O.D. SOLID NICKEL - 12" HEATERS
P = 248 PSIA (T SAT = 400.2 °F)
P = 93 PSIA (T SAT = 328.6 °F)
P = 27.2 PSIA (T SAT = 244.8 °F)
P = 4.3 ± .1 PSIA (T SAT = 155.3 °F)
Q/A (BTU/HR-FT²)
ΔT SUB, °F

FIGURE 3. Variation with pressure and subcooling of peak heat flux for boiling water at low velocity.

raise the heat flux beyond the peak. FIGURE 4 shows the data for a particular run with highly subcooled water.

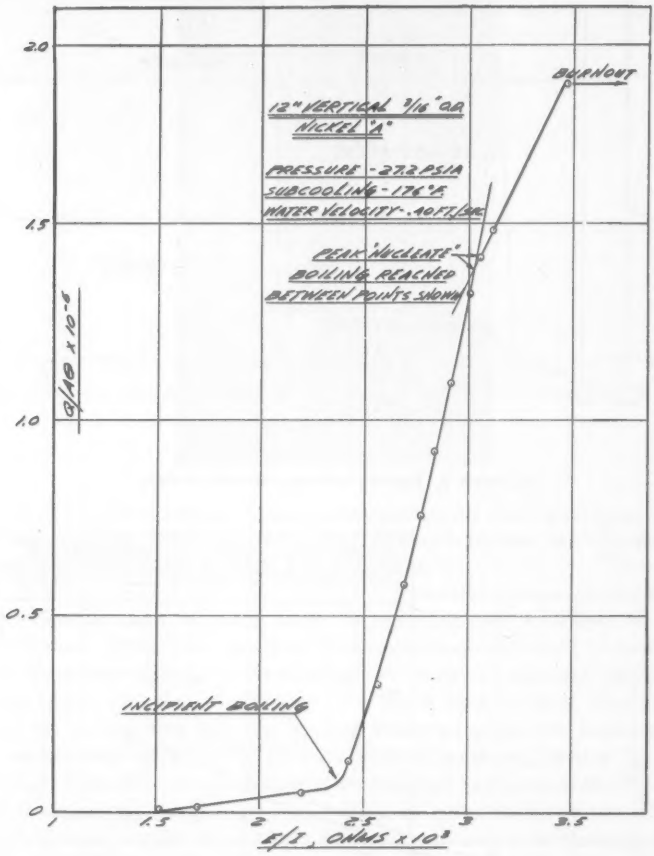

FIGURE 4. Typical "burnout" run with highly subcooled water and vertical nickel tube. Vibration develops at high heat fluxes.

FIGURE 5 shows microflash photographs of surface conditions in local boiling from about midway up the nearly vertical curve of FIGURE 2 up to the peak (see TABLE 1). Careful examination of photographs such as these and visual observation suggest that true nucleate boiling as described above occurs only over the lower two thirds of the steeply rising curve of FIGURE 2. As the peak is approached from the left, patches

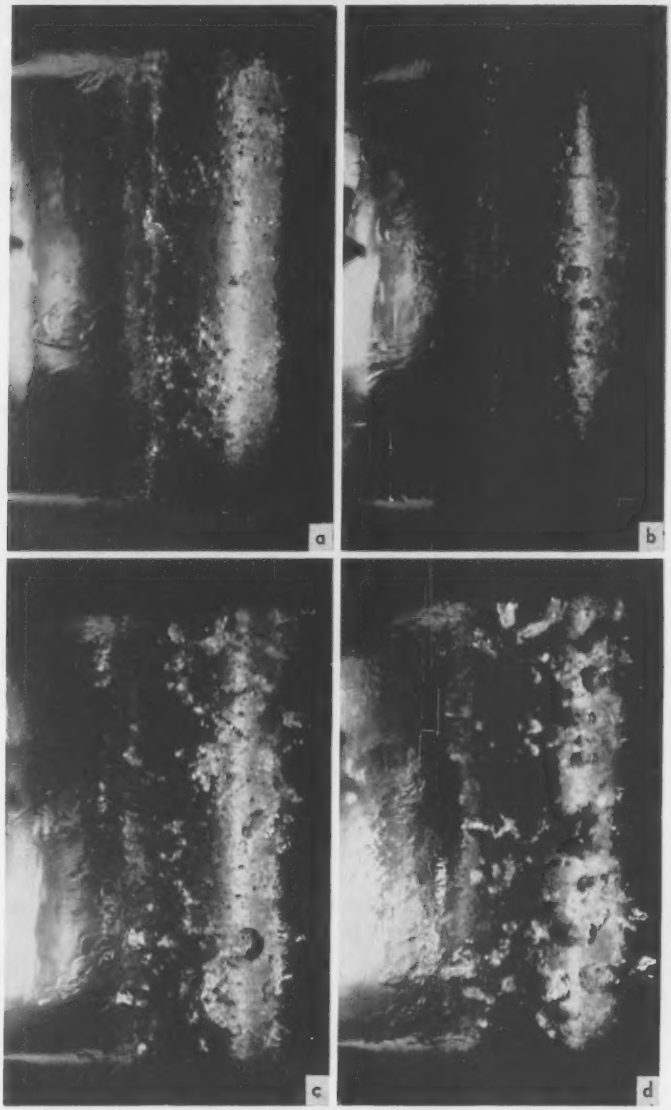

FIGURE 5. (a) Subcooled boiling (TABLE 1) 1,030,000 (B.T.U. per square foot) per hour. (b) Subcooled boiling (TABLE 1) 1,085,000 (B.T.U. per square foot) per hour. (c) Subcooled boiling (TABLE 1) 1,185,000 (B.T.U. per square foot) per hour. (d) Subcooled boiling (TABLE 1) 1,230,000 (B.T.U. per square foot) per hour.

resembling film boiling occur with increasing frequency while true nucleate boiling occurs elsewhere on the surface. In other words, a rapidly oscillating mixed regime appears to develop on the low temperature difference side of the peak. The film patches collapse quickly because the surface is then not sufficiently hot to maintain local evaporation at a rate sufficiently high to sustain the film. The patches seem to start because the rate of vapor generation by nucleate boiling is so great that outflow of vapor prevents inflow of liquid to the surface.

TABLE 1

Figure no.	*B.T.U. / (hr.) (ft.²)*	*Wall temp. °F.*	*Water temp. °F.*	*Pressure lb./sq. in. abs.*	*Sat'n. temp. °F.*
1	600,000	370	147	93.2	322.7
5a	1,030,000	380	150	93.2	322.7
5b	1,085,000	383	160	93.2	322.7
5c	1,185,000	383	163	93.0	322.6
5d	1,230,000	385	165	93.0	322.6

Burnout at 1,342,000 (B.T.U. per square foot) per hour.

In certain conditions with subcooled liquid, violent vibration develops as the normal nucleate boiling peak is approached. Measured peak is then abnormally high: vibration has facilitated heat flow into body of the liquid. One of the most interesting problems at present involves study of the boundary layer in the liquid through which, in local boiling, heat must flow to reach the main stream.

When boiling occurs in narrow passages, as in some nuclear reactors, formation of vapor may interfere with flow of liquid to the hot surfaces. In such cases burnout may occur simply because the cooling medium reaches the hot region chiefly as steam, which is unsatisfactory as a coolant. Because such circumstances are usually associated with an unstable condition of flow, this is called an instability burnout. Such instability is a serious hazard when many channels exist in parallel. It should be carefully distinguished from a true "boiling burnout."

THE NEW YORK ACADEMY OF SCIENCES

SECTION OF PSYCHOLOGY

THE ROLE OF PROPRIOCEPTION IN THE CONTROL OF BEHAVIOR*

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Introduction

Proprioception (kinesthesis, muscle sense, afferent feedback) has been a fundamental consideration in various systems or cults of body control. Today some therapists attempt to teach "a better feel of oneself," and behavior science, including reinforcement theory, appeals to "response-produced stimuli" to round out its account. Nevertheless, experimental data continue to be meager.

A basic technique of experimental psychology is to present stimuli to an organism and observe its response. The stimuli that affect exteroceptors can be specified and controlled with relative ease; consequently, vast literatures have accumulated with respect to vision and audition. For proprioception the case is different. Only the organism can present itself with proprioceptive stimuli. Thus this area of interest, although admittedly of crucial theoretical and practical significance, has necessarily seemed to be "private" and inaccessible to any genuinely scientific method of investigation.

Modern instrumentation suggests a possible point of entry. Muscles generate small voltages proportional to their contraction. Such action potentials, picked up at the level of microvolts by electrodes on the surface of the skin, may, after electronic amplification, have strength sufficient to permit them to be displayed on an oscilloscope or meter. In a sense, the electrodes "see" what the proprioceptors "see" and, most important, they do so in the public fashion that characterizes science.

The experimental subject, if allowed to watch a meter registering amplified voltage outputs from his own muscles, can correlate the degree of muscle contraction with the extent of meter deflection and, in effect, acquire a scale in terms of which he can report amplitude of private stimuli. As will be seen, he becomes able to perform this fairly well even when the meter, by means of which he learned the scale, is withdrawn from view. With the barrier of privacy breached by this stratagem, the way is open to scientific scrutiny of proprioception by standard psychophysical methods, by tracking procedures, and by other means.

*This paper, illustrated with slides, was presented at a meeting of the Section on May 19, 1958.

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In this discussion, however, the main emphasis lies, not on the method *per se*, but on its potential capacity to reclaim for experimental psychology areas of inquiry that in recent times have largely gone by default to the clinician. In particular, the method's relevance to a research program that has been under way for several years will be appraised and given a preliminary test. A statement of the background of that program and its rather devious progress to date follows.

The Problem of Behavior that Controls Other Behavior

I have been preoccupied with the problem of how certain control features become installed in man's behavior repertory by virtue of the socialization process that he alone, of all the animals, undergoes. Specifically, what is it that lends plausibility to the belief common to all human societies that the individual is responsible for his own behavior and can be held accountable for it? As scientists, we dismiss as fictional the doctrine of the person as prime mover. The organism's responses are necessary resultants of clusters of variables. Nevertheless, every day we do see people – some, at any rate – successfully resisting temptation, kicking against the pricks, and in diverse ways seeming to defy environmental pressures. What hidden variables make this possible?

Automation principles supply a clue. We design machines that run themselves unattended. Do we unwittingly invoke the same principles in conditioning the human young?

In the automatic machine we explicitly provide feedback from its own operation. For instance, the clock radio, once its switch has been set, moves a contact moment by moment closer to a second, stationary contact. By bringing them to the point of touching, it "reminds itself" to emit soft music. This is discontinuous feedback. The same radio also "knows enough" to keep the program from fading, for it not only amplifies but monitors the station signal. Should the signal grow weaker or stronger, the automatic volume control turns the gain up or down. This is continuous feedback.

The organism is well equipped to monitor its own behavior. Proprioceptors, embedded in muscles, tendons, and joints, supply feedback for position and movement. Their most obvious function is to modulate and coordinate responses that are predominantly under the control of exteroceptive stimuli; when interest focuses in stimuli administered by the experimenter, for many problems the proprioceptive contribution may safely be ignored. This is especially true in animal work, where care is taken to minimize individual differences of genetic origin by using subjects of the same strain, sex, and age – litter-mates, if possible – and where individual differences that would accrue from diverse reinforcement histories are obviated by using naive animals. If these

animals have been cared for in identical fashion until the time of their use in experimentation, the assumption is either that they have had no history or that they have had a common history. In this way proprioception, to the extent that it affects the data, does so only as one of the controlled variables.

In university laboratories, after serving in a single study experimental animals are "kicked out of school," not because of failure to learn, but because they have now learned too much. No longer naïve, they might begin to produce "self-instructions" of the sort that have been the bane of experimental work with human subjects.

In some animal studies, particularly those using rats or pigeons, proprioceptive stimulus control has not been wholly neglected but, as a matter of fact, has recently come to the fore under another guise (A. Weissman, 1958, unpublished data). The orderliness of results from various schedules of reinforcement depends upon the animal's coming to discriminate his own rate of responding, presumably on the basis of proprioceptive stimulation. Analysis of data, however, proceeds almost solely in terms of stimuli and conditions imposed by the experimenter, with proprioception mentioned, if at all, only in passing, and with the animal retaining the status of the inviolable "black box." The experimenters concerned are, of course, impeccably correct in restricting themselves to "the observables", and it is certainly true that the prediction and control of animal behavior it is certainly true that the prediction and control of animal behavior proceed apace soundly and steadily over broad areas without taking explicit account of stimuli internal to the organism.

To what extent the human organism can be dealt with experimentally on the same basis is moot. By the time he arrives in the laboratory he is not a naïve animal. He has a reinforcement history but, except superficially, this is unrecorded and unavailable. Motivation is not, except in special instances, aroused by depriving him of food or water, but by appealing to his good will, his competitiveness, or, in well-financed undertakings, his "money drive." His behavior is quickly brought within range by carefully worded instructions, not by turning him loose in the laboratory and shaping out successive approximations to the desired behavior.

It is not intended to imply that the human subject operates by psychological principles different from those of the subhuman animal, but simply to assert that in the course of his long, nonlaboratory-conditioning history a substantial part of the control originally exercised by exteroceptive stimuli comes to be shared by conditioned proprioceptive stimuli. In situations of most intimate importance to the person and derivatively to society, proprioceptive stimulus control may dominate the picture. It is suggested that it is this development that answers the question posed earlier – namely, why does it seem plausible to hold the individual responsible for his own behavior?

All this has been said before. What is different here is that it is taken to be, not the end of the matter, but the beginning of its explicit experimental investigation, where the criteria of progress will be the usual ones: increased ability to predict and control.

A promising place to start, with the phenomena in question seemingly present in relatively pure culture, is in the field of avoidance. The organism takes preventive action against dangers not yet in sight. This is adaptive and is conventionally glorified as "foresight." However, the organism may also defend itself against unreal contingencies or refrain from participation in situations which, to neutral observation, are most attractive. Neurotics and psychotics exemplify such behavior in the extreme, but it is also true that much human behavior not regarded as pathological is characterized by avoidance of dangers which, even in the absence of preventive action, would not materialize. Such behavior is "mistaken", as perhaps the individual himself will admit. If not controlled by properties of the present external situation, it must be attributed to properties of the present internal situation. The latter is hypothesized to include patterns of proprioceptive stimulation conditioned aversively in past situations and never extinguished.

A closer, more operational investigation of these matters than that offered by clinicians is sorely needed in a science of human behavior, but the technical difficulties are formidable. However, when confronted by a problem too complex or too inaccessible for direct study at the human level, an experimental psychologist may endeavor to study it in miniature or in analogous form with animals.

Proprioceptively Controlled Avoidance Behavior in Animals

In a monograph concerned with avoidance I reported in 1950 a study in which rats were conditioned to hold themselves seemingly immobile for long periods.[3] Light aversion was the drive employed. Keller[7] had shown that a rat, given suitable opportunity, will turn off a light. In the experiment now cited, as soon as a rat pressed down a small, movable bar, a bright overhead light went out. The bar press constituted an effective escape response from noxious stimulation. However, the switch was rigged so that when the animal released the bar the light immediately relit. In other words, bar-releasing behavior was negatively reinforced (punished).

Under these conditions the rat learned to hold the bar down without once releasing it for as long as 45 minutes. In the final stage of training the rat had to stand on its hind legs to reach the bar. Nevertheless, in the course of an experimental session lasting one hour it might not leave the bar and thus have light for more than a total of 10 seconds. After lengthy stretches of bar holding it is likely that muscular strain eventually became so punishing in itself as temporarily to override other

variables. The observed behavior was that the animal would release the bar, race about the cage for a few seconds, and then fling himself once more at the bar.

In the analysis of these prolonged "holds" it was assumed that the animal made incipient releasing movements. However, the least motion in the direction of bar release would presumably present discriminable proprioceptive stimuli to the rat. Such stimuli, since they always occurred just prior to the onset of aversive light, would become aversive (punishing) in their own right on the basis of simple Pavlovian conditioning. Therefore, any response that reduced or put an end to the now aversive proprioceptive stimuli would be positively reinforced (rewarded). Such a response might be a mere discontinuance of the release movement or, more likely, activation of antagonistic muscles.

Schoenfeld[11] has written the paradigm for the general case of avoidance as shown in FIGURE 1. S_1 is originally a neutral stimulus and S_2 a noxious one. However, if S_1 characteristically precedes S_2 it will become noxious also on a conditioned basis. In the holding experiment S_2 is the glaring light and S_1 the proprioceptive stimuli accompanying bar release.

The animal will now be motivated to take action sooner in the sequence of events. Whereas the rat previously worked to escape S_2, it will now, given the chance, work to escape S_1. In the second part of the paradigm R^T is a terminating response. The additional symbol (Av) is included to indicate that, in addition to terminating S_1, it also avoids S_2. The transverse bar shows that the correlation between S_1 and S_2 is broken. In the holding experiment $R^T(Av)$ is resumption of bar pressing. This simultaneously escapes the conditioned aversive effect of the proprioceptive stimuli, thus immediately reinforcing the response, and it also has the derivative effect of avoiding S_2, the bright light.

So far the animal's behavior is adaptive, but will it extinguish readily when the bright light is no longer contingent? Under extinction procedure

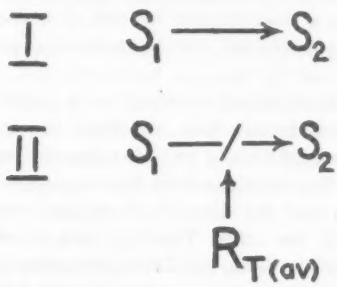

FIGURE 1. Schoenfeld's paradigm for the general case of avoidance (see text).

the rat was introduced into a darkened cage. When, in wandering about, the animal happened to depress the bar, it placed itself in the precise situation that had prevailed during conditioning—that is, the light was out and it was holding the bar. As a result, for several hours of extinction procedure the animal actually spent nearly as much time holding down the bar as during conditioning. Later the amount of holding tapered off, but even after prolonged cessation the rat's bar pressings were of substantially longer duration than they had been prior to the training in holding. The proprioceptive stimuli attending bar release apparently still exerted considerable control.

The apparatus was not designed to permit measurement of incipient bar-releasing movements or, for that matter, even to demonstrate that they occurred. In a follow-up study by W. Winnick (1949, unpublished data), the animal, instead of pressing a bar, pushed against a small vertical panel hinged at the top, The panel could be pushed further than necessary to turn off the light, and a pen writing on a moving paper tape indicated extent of movement. Another stationary pen drew a base-line that showed the position of the panel with the light on. Early in conditioning the animal wavered back and forth, frequently allowing the light to return momentarily. Later, the light was kept off for long periods, but the panel pushing was not "all-or-none." The recording pen revealed a drift toward the position where light would be reinstated but, before this occurred, the animal would make a corrective thrust back to a "safe" position.

While Winnick's study demonstrated clearly that the holding response is one of continuing activity, with incipient movements occurring and then being corrected for, it was still merely a matter of inference that the proprioceptive stimuli set up an aversive or emotional or "anxious" state in the organism. To test for this objectively L. O. Eldridge (1954, unpublished data) designed a variation of the experiment that permitted respiration measures to be taken. Breathing has long been regarded as a sensitive index of autonomic upset. While this investigator did not succeed in devising a pneumograph for rats that would be reliable with respect to breathing amplitude, he did solve the problem with respect to breathing rate.

Eldridge's rats were trained to stand on a perch several feet above the floor. This kept the rat from attacking the soft-rubber pneumatic bulb strapped around its middle and also forestalled light-reducing maneuvers other than the one chosen by the experimenter. Two small rods were positioned so that the animal's front feet were supported by one and his hind feet by the other. Teaching rats to behave to this extent like birds was time-consuming, but Eldridge reports that during the course of actual experimentation only one animal ever fell off his perch, and that one only once.

Directly over the animal's head was a wooden block, counterweighted to rise if the animal exerted an upward push of about 5 gm. Its slightest elevation turned off 2 unfrosted tubular lights, suspended on either side of the rat.

The animals readily learned to stand with their heads in the slightly up-tilted position that removed the light. It was expected that, if the incipient movement of withdrawing actually produced "anxiety", it might be possible to demonstrate accelerated breathing. Eldridge accordingly analyzed his records for breathing rate just before and just after the beginning of movement in the direction of head withdrawal.

His control records of breathing in a free situation had indicated that rate changes continuously. Rate per minute of any two breaths, checked against the previous two, is, with equal likelihood, greater, less, or the same.

The first time a rat removed the light by lifting the block and then allowed it to relight again by withdrawing his head it was not expected, of course, that any acceleration would occur, and it did not, beyond the probability to be expected by chance. However, it did occur *without exception* and without doubtful cases on every single subsequent withdrawal or partial withdrawal during this and later conditioning sessions. The number of instances measured totaled more than 1200. If the breathing rate before incipient head movement was about 80 per minute, then the first two breaths after movement were likely to be more shallow, although not necessarily, and the rate anywhere from 100 per minute to—although this was rare—400 per minute.

That accelerated breathing was not simply an invariable concomitant of movement itself was demonstrated by the fact that in extinction, when light could no longer be escaped or avoided, the acceleration disappeared on most head withdrawals. At the end of the extinction procedure, which totaled 7 hours, accelerated breathing had not disappeared in all instances, but was down from 100 per cent to 40 per cent, but it was either there or not there. Extinction did not give rise to doubtful measurement cases.

These three experiments comprise a unit. I conditioned the holding response and gave an inferential account of the behavior processes involved. Winnick followed with a demonstration that the holding was not immobilization but vacillation between release movements and compensatory renewals of forward thrust, and Eldridge finally established the presence of an aversive state or "anxiety" set up in the animal by its own proprioceptive stimuli.

Reconsideration of Avoidance at the Human Level

The dynamics of avoidance were somewhat clarified by the animal experiments just cited. Further inspection of the human situation sug-

gested that the young child undergoes loosely controlled but intensive training in a variety of avoidances. He is punished for bad behavior. Traditionally it has been supposed that this should annihilate further tendencies to such behavior, but evidence has accumulated indicating that punishment does not expunge responses from the repertory. Dinsmoor[2] has reviewed the experimental literature on the subject.

Punishment's effect is, rather, to condition a holding-back response. The punishable behavior, indeed, may be effectively restrained from overt manifestation, but it is not extinguished and, in many cases, it is not replaced by alternative responses more acceptable to parental or other social authority. Instead, there appears to develop a kind of low-grade, chronic deadlock comparable to the conflicts described in animals. Whole areas of behavior apparently may be affected, particularly those that constitute sexual behavior, aggression, or even, in some families, merely "getting excited."

From animal experimentation we know how slow avoidance responses may be in extinguishing. We might suspect that exceptionally severe punishment in childhood could set up avoidance patterns not extinguishable in a lifetime. To the extent that the punished behavior was and is definitely antisocial, such a training method might be deemed unfortunate but necessary. This was probably the position of Freud when he stated that "repression is the price of civilization."

Much of what is punished in childhood, however, seems to be merely the precocious appearance of behavior not only sanctioned in the adult but actually demanded of him. For a child to be thoroughly conditioned either to accept unquestioningly or to resist blindly what may be arbitrary (or, indeed, nonsensical) parental demands suggests that, although later he might meet the criteria of maturity in many conventional respects, he would remain overrestricted in his behavior. His effectors would chronically be partially engaged in restraining behavior now appropriate to adult status. This would be the situation clinically called "residual tension."

Should this be the predicament of a sizable portion of the adult population, it would not be surprising to find well-nigh universal complaints of aches, pains, cramps, tension, and "run-down feeling," or that Edmund Jacobson[4,5] should have had a best-seller on his hands when he wrote *You Must Relax*. If such symptoms arise from behavioral blocking of the type described above, then it should also not be surprising that Jacobson's system of progressive relaxation should have only limited efficacy. With avoidances of substantial strength involved, the patient could not afford to relax because of the intensity of the anxiety that the relaxation process would generate in such cases.

Malmo[6] and his co-workers have investigated electromyographically headaches produced by chronic contraction of the musculature of head and scalp. They find these related to unexpressed resentment. They

also suggest that leg crampings of a certain kind may be related to sexual inhibition, and arm and shoulder crampings to the inhibition of aggressive behavior. A general review of this subject has been made by Plutchik,[10] who proposes as a new criterion of normality "the absence of residual muscular tension."

Modern psychotherapy's approach to the problem is a verbal one, The patient is encouraged to talk about what he has previously held back. At intervals the therapist, depending on his school of thought, may make interpretations, ranging from shrewd remarks about specific behavior to dogmatic pronunciamentos. When effective, the process would appear to be so in part as a result of extinction of conditioned aversive states, a necessary price being the toleration of anxiety as the patient receives encouragement to avoid making his previous avoidance responses. When ineffective, such verbal therapy might merely add to the patient's conversational repertory, giving him encyclopedic knowledge of his symptoms and their theoretical meanings without other marked changes in behavior. In such cases verbal therapy would have had only verbal effects.

In the context of a science of behavior the question arises as to whether the investigation of chronically avoidant behavior, as hypothetically formulated above, might not be undertaken directly – not by way of talk and inferred content of conflicts – but by way of proprioceptive discrimination of actual muscular aspects of the blocking. This would take the condition of the musculature to be part of the world of things and events and susceptible to observation and verbal report.

Verbal Report of Proprioceptively Induced Blocking

Mimeographed instruction sheets entitled *Informal Experiments in Self-Awareness* were prepared and distributed to undergraduates enrolled in courses in abnormal psychology. The students were required to submit written reports of results personally obtained in carrying out various procedures. The instructions were revised and expanded over the course of several years, and sets of reports have now been collected from more than one thousand students. An early version of the material, with verbatim excerpts from students' reports, was published in 1951 as the first half of a collaborative book rather misleadingly entitled *Gestalt Therapy.*[9]

As part of his assignment the subject was encouraged to make what amounted to a systematic proprioceptive survey of his own body, conducted in private with minimum external distractions. A person neither dead nor in flaccid paralysis presumably should be able to discriminate the tonic condition of all or of any part of his skeletal musculature. The first report of a subject is likely to be that he can do this. He can feel, he says, every part of his body. When further inquiry is made, it often turns out that what he took to be proprioceptive discrimination of a particular body part was, however, actually a visualization of the

part or a verbal statement of its location. Or else, to discriminate the part, he may have had to amplify proprioception by making actual movements.

With further work, if he can be persuaded to continue, the subject may report certain parts of his body to be proprioceptively missing. Suppose it is his neck. He may discriminate a mass that is his head and a mass that is his trunk, with what feels like simply some empty space between. At this stage the subject is apt to remember more important things to do and his participation in the silly business ends.

Some subjects, however, apparently made curious by blank spots and hopeful of recovering some lost degrees of freedom in their system of voluntary control, do whatever is involved in paying closer attention to and acquiring interest in this peculiar private situation. A blank spot, they say, may gradually fill in. Or it may suddenly become the locus of sharp pain, paresthesias of one sort or another, "electric" sensations, or the unmistakable ache of muscular cramp.

Then what formerly was a blank may become as demanding of attention as an aching tooth. Further and more detailed discriminations may be made. It soon becomes imperative to relax the cramp, but the subject says that he does not know how to do so; he is concerned with so-called voluntary muscles, but these are reportedly not under voluntary control. The subject is somewhat in the position of the elementary psychology student who is assured that he has the voluntary muscles needed to wiggle his ears. The difference, of course, is that in the case of ear-wiggling he has never acquired control whereas, in the case of the cramped muscles, he has somehow lost control.

As soon as the question becomes one of how to acquire or regain control, problems for investigation sprout in all directions. A host of variables, direct or indirect in their effect, becomes relevant. One involves merely the instruction to continue to pay sustained attention to the blocking, regardless of discomfort, and to be on the alert for subtle changes of any sort. This seems to give rise to what previously was called ideomotor action, and may in itself bring a loosening of the reported muscular clinch.

Another instruction is to increase the clinch deliberately, if possible, and then, while relaxing from this added intensity, to learn something about relaxing still further. Also relevant are procedures that make use of proprioceptive facilitation – for instance, those used in training polio victims, partial spastics, and others to make better use of whatever healthy muscles they still possess. Kabat[6] has developed these methods systematically.

When a muscular block is definitely resolved, it is frequently claimed by the subject that there occurs vivid, spontaneous recall of typical situations, perhaps dating back to childhood, where he learned to tense in this particular manner. Should a correlation between recovered move-

ments and recovered memories be found and turn out to be high, it would afford substantial support to a motor theory of the so-called higher mental processes. It would generalize in ways not clearly foreseeable Davis's[1] basic experimental finding that the "stimulus trace" is conserved in the differential activity of muscles.

Such subjective accounts of proprioceptively controlled events, although they may be revealing and fully convincing when made at first hand in observing one's own behavior, are obviously not to be taken at face value as scientific data. They have merely introspectionistic standing unless or until they can be corroborated objectively in the laboratory. A first step in this direction, it is believed, is the following attempt to achieve quantitative measurement of proprioceptive stimuli and to lay foundations for a method of checking verbal report against instrumentally obtained data.

Apparatus

Since the microvoltages picked up from the subject in electromyographic work are of the same order of magnitude as radio and television signals, it is usually necessary to enclose the subject in a space where relative electric silence has been achieved. A grounded wire-mesh cage may be sufficient for this purpose. The enclosure constructed in the present instance was 4 feet wide, 8 feet long, and 8 feet tall, large enough to contain a folding-type posture chair and to house the early-stage amplifiers.

However, due to the fact that the room available for laboratory use was adjacent to one housing heavy solenoids and relays and in a building containing many types of radiating equipment, it was necessary to work out empirically additional means of noise reduction. The more important were a filter system for the power line and the operation of all but the last stage of amplification on batteries.

The masseter (jaw muscle) was selected to record from because of its favorable innervation ratio, accessibility, and previous training through involvement in such activities as eating and talking. Three electrodes were affixed to the subject. Two were dime-sized silver discs, one placed at the angle of the jaw over the belly of masseter and the other over the temporal bone just far enough back to avoid eye-blink artifact. The third electrode (ground) was a one-quarter-inch German silver disc soldered to an earring screw and tightened securely on the subject's ear lobe. The right side of the face was used with all but one subject.

The skin was rubbed with acetone-soaked, rough paper toweling to remove dead cells and oils. Sanborn electrode jelly was then rubbed in at the precise spots for electrode placement. The two recording electrodes were attached and held tight to the skin by 2-inch squares of Hy-Tape surgical adhesive, slit for emergence of the leads.

The leads terminated in phone tips and were just long enough to reach easily to a multiple plug-in box strapped to the subject's left arm. From that point a shielded cable connected to the first amplifier on a shelf behind the subject's chair.

The initial two stages of amplification were operated push-pull, permitting rejection of in-phase potentials from the recording electrodes and thus completing the work of attenuating extraneous noise. From this point on all amplification was single-ended – not by choice, but because of the limitations of the equipment.

Three Tektronix Type 122 low-level preamplifiers, each set at a nominal gain of 100, were cascaded to provide a combined gain of approximately 100,000 (most of the apparently available gain was sacrificed in impedance-matching resistance networks between amplifiers). The signal was then conducted outside the electrostatic cage and fed to a Viking RP 61 amplifier to reach a final amplification of 1,000,000.

A portion of the fully developed signal went to one channel of a Viking tape deck and was magnetically recorded. It was intended to facilitate the processing of data by playing back the muscle action potentials through a voltage integrator; however, at this writing the technique has not been completely worked out, as there has remained some difficulty in assuring strict linearity of the playback voltages. As a result, data reported here were obtained by a more laborious method from what had been intended to be merely a monitoring or quick inspection device.

This was an Esterline-Angus recording milliammeter, adapted to serve as a recording voltmeter via the mediation of an RCA vacuum tube voltmeter (WV-98A). A second portion of the signal was fed to this VTVM, operating on its 0-5 v. scale. With an input of 5 v. the meter circuit carried a rectified current of 200 μamp. The meter itself was removed from the VTVM and put to a use that will be described shortly, while the current that would ordinarily go to the meter was conducted to a Tektronix Type 112 DC amplifier. Here it was boosted from 200 μamp. to 1 ma., enough to drive the pen of the recording milliammeter to the full width of the paper roll chart.

Still a third portion of the signal went to a second VTVM, and fluctuations in signal strength from 0-5 v. were observable on its dial. Since the experimental design required, however, that the subject be able part of the time to see the metered output of his own muscle, a switching arrangement was devised to transfer at desired intervals the meter current of this second VTVM to the spare meter mentioned above, now housed in an aluminum box on an adjustable support directly before the subject's eyes (FIGURE 2).

Gain determinations were made by putting known signals through the system. These were supplied by a Heath sine-square generator (AG-10) passing 75 cps sine waves through a General Radio microvolter.

Programming equipment may perhaps best be described in the following section.

Subjects and Experimental Procedures

Five young men and one young woman served as subjects. Two were graduate students in psychology, the others were undergraduates. Before their first recorded session they were told the nature of the experiment and, if interested, shown the equipment.

Once the electrodes were in place the subject was tilted back in the posture chair to a semireclining position. In an earlier pilot study an ordinary armchair was used, but it soon became obvious that readings from the jaw muscle were highly sensitive to such factors as postural adjustment and gestures. To minimize such effects an adjustable foam rubber headrest was constructed, as well as a special arm support, and microswitches were wired to activate a buzzer and a warning light whenever the subject changed from the standard position. In the present study it was hoped – and it did turn out to be the case – that such cumbersome precautions would be unnecessary with the subject relaxed in a reclining chair.

Overhead lights were turned off, leaving the room softly illuminated by several shaded lamps. One light shone directly on the subject's meter, but this meter was not switched on until the experimental run began. Meantime, the subject's average action potentials were appearing on the experimenter's meter and were being recorded by the ink writer (the meter was critically damped to give peak-to-peak readings and thus presented none of the objectionable jitter that would have occurred otherwise).

The next step was to arrange the meters to read zero at the subject's resting level, which was not, of course, zero signal strength. The first amplifier produced, according to the manufacturer's calibration report, nearly 2 μv. of noise, or, with a gain of 1 million, 2 v. on the meter, and the subject himself, at his resting level with jaw sagging open, contributed another 2 or 3 μv. (in the case of one subject much more). With the meter on its 0-5 v. scale this meant that the needle would be deflected nearly full scale before the subject did anything at all by way of contracting the masseter.

The problem was met by running a bucking current – that is, one of reverse polarity – through both meters (the indicating meter and the one that drove the recording pen). The bucking current was supplied by a 1.5 v. mercury cell through a series of rheostats and was introduced in an amount just sufficient to back off the existing meter reading and leave the needle hovering about zero. Later, when the subject was instructed to close his jaw slightly, the full range of the dial was thus available to register his action.

Before starting the first experimental session the subject was instructed as he lay relaxed with jaw sagging: "We are now about to begin. In a short time you will hear a 'beep.' At the same time a light will

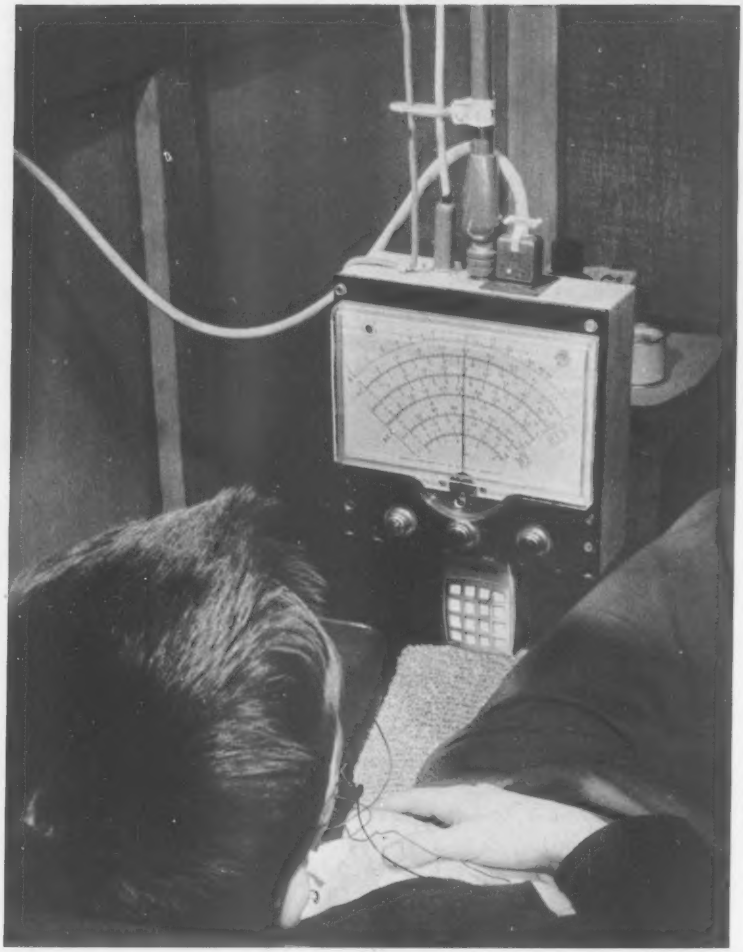

FIGURE 2. Subject and experimental arrangements viewed from rear of electrostatic cage.

appear on the left side of your meter case just below the dial. This means that you are to go to work. What you are to do is to close your mouth by the smallest amount you can. This will make the needle rise on the scale. It will take only the tiniest bit of movement from you to make the needle go to the top of the scale – so little, in fact, that no one watching you would be able to say that you had moved at all. We

want you to try to make the needle go to midscale – to the position where it is standing straight up – and then try to keep it there.

"After you have done this for 10 sec. you will hear another 'beep.' The middle light will come on, and your meter will go dead. The needle will drop down to zero and stay there. But what you are doing with your jaw will now show up on a meter just like yours that we have out here. What we want you to do during this 10 sec. is to keep *our* meter at midscale with the needle standing straight up – even though you can't see it!

"Then the 'beep' will come againn and the light on the right will go on. This means that you are in a 10-sec. rest period and can do whatever you want – move around, say something, stretch, anything at all.

"Just before the end of your rest period you will hear a double click. That's the counter out here recording the number of the cycle just ending. Right after that the left light will come on again and a new cycle will start. There will be thirty of these cycles in all. They will take 15 minutes."

Questions by the subject were then answered and portions of the instructions repeated as needed. For the first two or three cycles on the first day the subject was given step-by-step reminders of what to do.

The experimental run began with the switching on of automatic programming apparatus. Two Industrial Timers were employed, one having its synchronous motor geared at 2 rpm and the other at 6 rpm. The motors drove sets of cams with adjustable notches, and these notches opened or closed circuits through the contact arms of microswitches.

Five cams were driven at 2 rpm. Three of these operated the 10-sec. lights on the subject's meter case, the fourth controlled the 10-sec. presentation of the active meter to the subject, and the fifth energized the cycle counter. Two cams were driven at 6 rpm. The first completed the circuit of the small oscillator that produced the beep, and the second operated a marker pen in the left margin of the roll chart used by the Esterline-Angus recording pen.

Since the three phases of each cycle were indicated to the subject by individual lights on the meter case, the beep constituted, so far as he was concerned, simply an additional, auditory notification of change of phase. Its major function was to put a time signal on the second channel of the stereo tape recorder, so that, in the projected playback of muscle potentials through the integrator, no synchronizing problems would arise. The second tape channel was also used to record such data as the experimenter's instructions, remarks by the subject, and equipment adjustments made during a run.

Two microphones were employed. One hung at convenient mouth level for the experimenter as he sat on a stool behind the cage, and the other was suspended from the subject's meter case. A selector switch made either immediately available. After an experimental run, when the experimenter entered the cage to remove electrodes from the subject, the latter's

microphone was cut in and an effort made to obtain a verbal report of any special strategies resorted to, opinion of progress, and whether the subject suffered from fatigue.

The above procedure is summarized in part A of TABLE 1. Three subjects were assigned to this procedure, although data from Subject 1 alone will be reported in detail. This subject worked on this schedule for 30 cycles per day, almost daily, for a total of 14 sessions.

Three additional subjects spent their first day on this schedule (only results from Subject 4 will be reported in detail). Beginning with session 2 they were instructed to attain and maintain 2 additional meter readings. These were 1.5 and 3.5 v. (midscale corresponded to 2.5 v.). The schedule for a particular experimental session was prepared by randomized selection of one of these three values for each of the thirty cycles. Then, just before the beginning of phase 1 (Vision-Proprioception) of each cycle, the experimenter called the task—for instance, "The value is one and one half." During phase 2 (Proprioception Only) the subject attempted to retain the assigned value, whichever it was, through the 10-sec. period without visual feedback from the meter.

TABLE 1

SUMMARY OF EXPERIMENTAL PROCEDURES

I. Single meter value: 2.5 v. (midscale)					
Phase	*Duration*	*Condition*	*Sessions*	*Subject's meter*	
1	10 sec.	VP*	All	On	
2	10 sec.	PO†	All	Off	
3	10 sec.	Rest	All	Off	
II. Multiple meter values					
Phase	*Duration*	*Condition*	*Sessions*	*Meter values*	*Subject's meter*
1	10 sec.	VP*	1	2.5 v.	On
			2 to 7	1.5, 2.5 or 3.5 v.	On
2	10 sec.	PO†	1	2.5 v.	Off
			2 to 3	1.5, 2.5 or 3.5 v.	Off
			4 to 7	1, 2, 3 or 4 v.	Off
3	10 sec.	Rest	All	—	Off

*Vision-Proprioception
†Proprioception Only

Beginning with the fourth session under procedure 2 another change was introduced. During phase 2 (Proprioception Only) the subject was required to attempt meter values never previously worked on—that is, they were never worked on during phase 1 (Vision-Proprioception). These were 1, 2, 3, and 4 v. They, too, were selected and presented randomly. At the instant when phase 1 shifted to phase 2, the experimenter called, for instance, "Give me four."

A total of seven sessions was conducted under this more elaborate second procedure.

Results

Since an increment of only 5 μv. from the subject's resting level sufficed to drive the meter full scale, it was foreseen that the needle would frequently peg, particularly while the situation was still novel, since, ordinarily, an instruction to a subject that he respond suggests to him that he make some gross, overt response. Meter-pegging represented no serious threat to the instrument, since it was electronically protected against burn out, but rather to the securing of measurable data.

The subject could have received preliminary training at a lower sensitivity level, but this would have accustomed him to a proprioceptive scale of coarse units. Furthermore, it would have permitted him to touch his teeth together and obtain discriminative stimuli from modalities other than proprioception.

Instead, what was done was to emphasize to the subject beforehand the subtlety of the response desired. Even so, all six subjects immediately pegged the needle in their first attempt to reach midscale. Some expostulated, "I didn't move my jaw—I just *thought* of moving it!" Apparently "smaller thoughts" became quickly available, in view of the fact that all but one subject within a matter of one or two cycles eliminated wild meter swings and settled down to the experimental working range.

A reproduction of the pen record for cycles 12 and 13 of session 1 for Subject 1 (male graduate student) is presented in FIGURE 3A. The excursions of the pen continue to be wide, as at the start of the run, but much learning has already occurred. The subject swings far past midscale with each adjustment of the masseter, but he is finding the range in which to work, so that the middle of each excursion tends to be near midscale.

It will be noted that during Proprioception Only (indicated in the figure by the small letter *b*) the subject for 5 sec. or more behaves much as he did with visual feedback, but then goes upscale and pegs, and remains pegged during most of the rest period.

Behavior during the second half of Proprioception Only was not consistent with respect to subjects, nor for the same subject at different stages of training. The usual tendency early in training was to slide downscale during the period without the meter and to drop to values around zero during the rest interval. This was true of Subject 1 during intermediate stages of training.

A second sample from the same subject during session 14 (final) is presented in FIGURE 3B. Its clarity is marred by artifactual pen swings at the end of each 10-sec. phase. These were produced by noise from the coil operating the pen that made time markings in the left margin

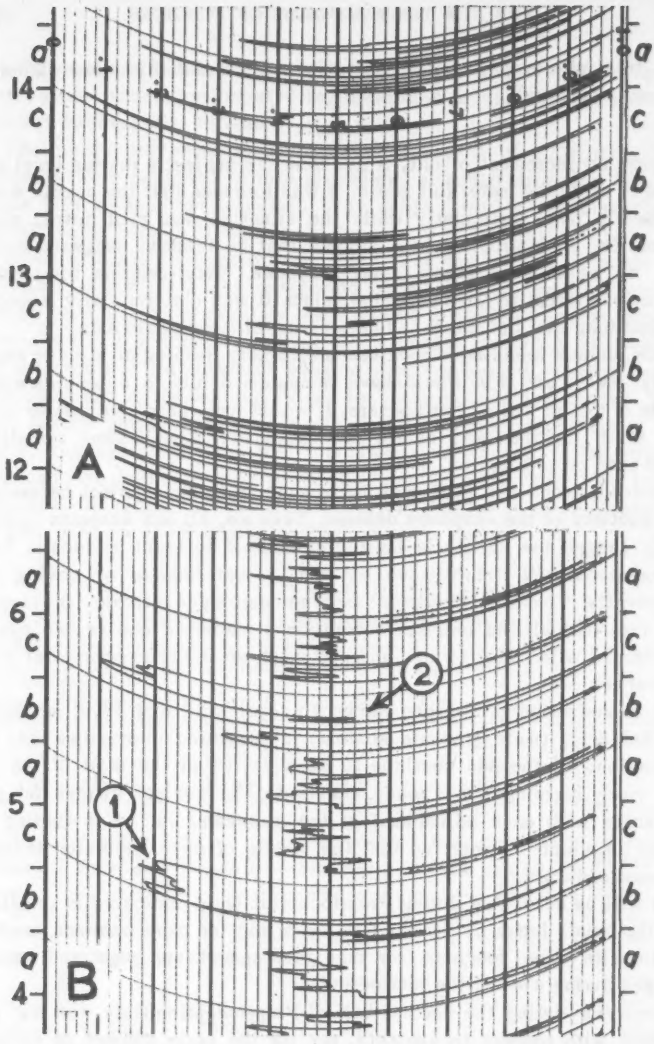

FIGURE 3. (A) Sample recordings from first and (B) fourteenth 15-min. daily session of training one male subject to keep meter at midscale by slight adjustments of jaw-muscle tension. Cycle beginnings are numbered at left, with phases—Vision-Proprioception, Proprioception Only, and rest—lettered *a*, *b*, and *c* between 10-sec. time markers in each margin. The scale is 0 to 5 v., corresponding to approximately 0 to 5 μv. at the skin electrodes. In B arrow 1 indicates continuation of work behavior into rest period and arrow 2 tendency of subject to stop for a few short oscillations about midscale as he swings past it in rest period.

(right-hand markers shown in the samples were hand-drawn). These artifacts did not contaminate the data, since they were readily recognizable and came only at instants when the subject's task was shifting.

Several effects of training are apparent. Midscale is closely approximated both with and without visual feedback. Also, the amplitude of oscillations has shortened. When it is remembered that full scale represents only 5 μv. at the skin electrodes, it may be seen that the total extent of the pen excursions during both working phases of the cycle is of the order of 1 μv. Beyond that, there are intervals, about one a second in duration, when the oscillations are no greater than 0.1 μv. The sample presented is in no way exceptional. Others might have been selected in which the pen virtually stands still for short intervals and where it appears that the subject is discriminating muscle changes that, as reflected at the electrodes, are of the order of one one-hundred millionth of a volt!

As training proceeded, it became noticeable for all subjects that activity during rest periods became patterned in a manner similar to that of the work periods. With onset of the rest period the subject tended to drop downscale somewhat and then to continue the small oscillations that had become characteristic of his efforts to attain and maintain some fixed value. Arrow 1 in FIGURE 3B points to such an instance. This carry-over of the "set" of the work period was obscured or destroyed during those rest periods or parts of rest periods when the subject made observable shifts of posture.

When asked what he did during rest periods, no subject displayed any awareness of this aspect of his behavior. Leading questions were carefully avoided until the termination of the last experimental session but, even then, when questioned point-blank about the matter, the subjects had nothing to contribute.

Another aspect of advanced stages of training, seemingly automatized, is indicated by arrow 2 in FIGURE 3B. Shortly after this rest phase began the subject made a movement that briefly pegged the meter. A moment later he relaxed to a point close to zero. However, on passing midscale he stopped for two quick, relatively short oscillations before proceeding. This, too, was a matter on which subjects, even with direct questioning, gave no verbal report.

The records of Subject 1 were submitted to detailed measurement, each sweep of the pen being taken as a behavioral unit. The 50 printed lines of the roll chart permitted convenient use of a scale of 0 to 100, and top and bottom values for each excursion were determined. Rest periods were not measured; after session 1, only the excursion end points in every other cycle were measured.

Decrease in average length of excursions might have been tabulated and plotted as a measure of increasingly fine differentiation of the muscle-adjusting response. However, while development of ability to restrict

overshooting and to make quicker and more subtle corrections presumably does indicate sharpening of proprioceptive discrimination in the sense that the subject is responding to smaller increments or decrements in jaw tension, the main interest here was in the accuracy with which the subject could produce a designated scale value both with and without visual feedback. The skillful small oscillations could be and frequently were made in a radically erroneous part of the range. For instance, during session 13 Subject 1 for 12 consecutive cycles produced values approximating midscale with small deviations during the visual feedback period; then, when his meter went dead, he dropped precisely 5 divisions downscale (0.5 v.) and worked, during the whole Proprioception-Only period, with exquisite control about that false value. After those 12 cycles the consistent downscale shift abruptly disappeared.

It was decided that scale-value discrimination could best be assessed by a constructed measure—excursion midpoint. Accordingly, the length of each excursion was determined by subtracting the bottom from the top value, dividing by 2, and adding this figure to the bottom value. Mean values by session for Subject 1 are listed in TABLE 2 and plotted in FIGURE 4.

TABLE 2

MEAN VALUES OF PEN-EXCURSION MIDPOINT BY SESSION FOR SUBJECT 1, AND STANDARD DEVIATIONS FOR PROPRIOCEPTION ONLY

Session	*Vision-Proprioception*	*Proprioception Only*	
		Mean	*S.D.*
1	49.19	27.38	12.91
2	46.22	31.00	7.34
3	49.90	38.70	7.07
4	53.12	48.06	8.35
5	53.07	54.60	8.20
6	55.00	47.58	8.71
7	57.00	51.18	6.94
8	52.85	51.82	13.25
9	53.85	54.46	10.80
10	54.16	46.96	4.52
11	49.84	52.97	8.00
12	52.02	51.59	6.79
13	50.18	42.98	4.88
14	49.80	45.80	6.90

Performance with visual feedback shows no improvement. This is taken to mean that the requisite skills were already highly developed and effectively brought into play during the first few cycles. During early sessions pen excursions tended to be long, but their midpoints were close to midscale. Each plotted point is based on from 150 to 200 separate excursions. Deviations on either side of 50 thus represent

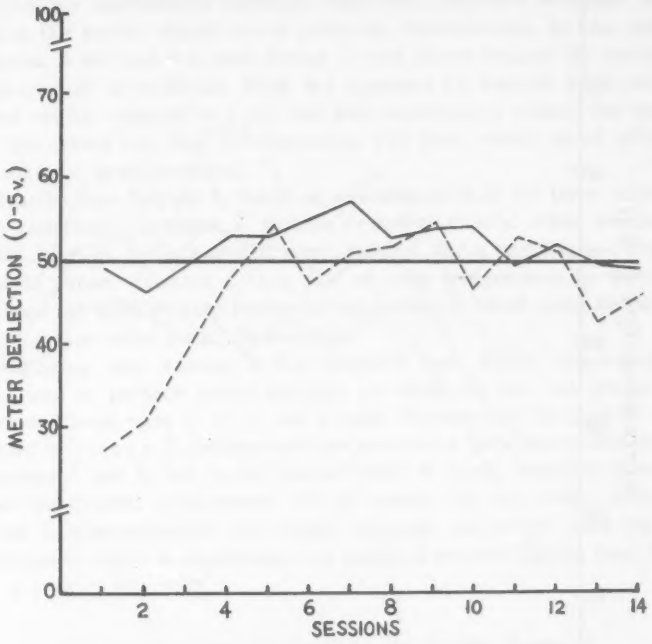

FIGURE 4. Mean values of pen-excursion midpoint by sessions for Subject 1. Solid line is for Vision-Proprioception and dotted line for Proprioception Only. Horizontal line at 50 indicates meter midscale.

rather consistent over- or undershooting during a particular session. The variables determining these effects remain unexplored. It may be recalled, however, that these deviations, while looming large in the plotted figure, represent extremely fine behavioral differences.

Performance under Proprioception Only began with marked undershooting, gradually corrected on succeeding days. By session 4 performance is indistinguishable from that under visual feedback and remains so thereafter. This does not suggest, of course, that sight of the meter has become irrelevant, but rather that, once given the range by visual feedback, the subject can maintain it during the short period required in this experiment. Some subjects boasted that they had acquired "absolute pitch," but it has not yet been convenient to test this claim.

In FIGURE 5 the means for Proprioception Only have been replotted within an envelope representing plus or minus one standard deviation. No consistent change in variability is observable. Standard deviations for Vision-Proprioception (unplotted) are of the same order of magnitude and also without systematic trend.

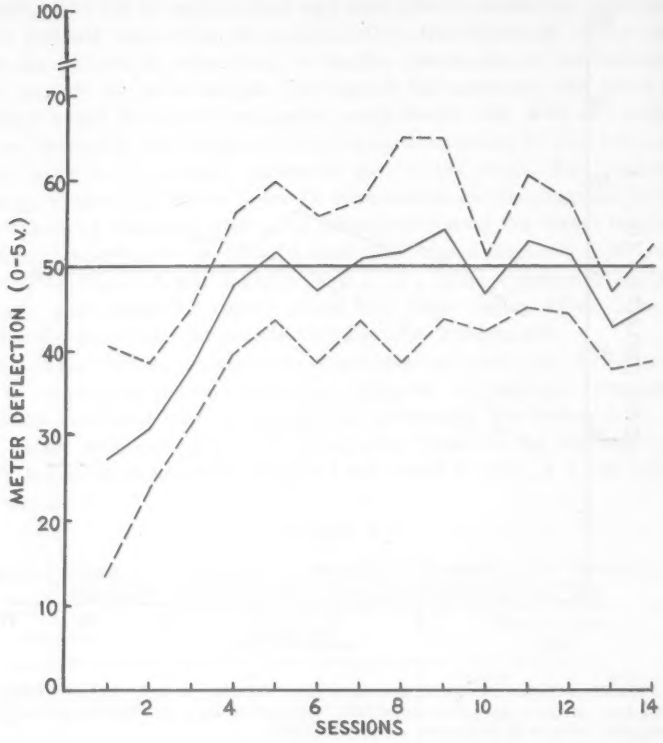

FIGURE 5. Mean values of pen-excursion midpoint by session for Subject 1 for Proprioception Only. Means are represented by solid line. Dotted lines indicate 1 SD above and below the mean.

Subjects 2 and 3 underwent the same experimental procedure. Their pen records have been inspected and appear different in no conspicuous way from those of Subject 1. Further processing of their data has been reserved until such time as the voltage integrator is available for this purpose.

Subject 2 (female undergraduate) differed from the others in having a high resting level for the first three sessions. In contrast to 2 or 3 μv. for them, her level was 18 to 20 μv. The bucking current available was not quite sufficient to back this noise off the meter, so that the only way to zero the meter was to reduce amplification slightly.

Her behavior during these sessions was anomalous. During rest periods she might peg the meter, but during work periods it seemed that she construed the task to be that of maintaining a zero reading. The instructions were repeated without effect. Upon request, she herself re-

peated the instructions correctly, and even indicated with her finger where the needle should be at midscale. Nevertheless, by the end of session 3 she had not once during a work phase caused the needle to rise as high as midscale. When she appeared for session 4 her tension level readily reduced to 5 μv. and she proceeded to control the needle as the others had from the beginning. Her later verbal report afforded no clues to an explanation.

Results from Subject 4, taken as representative of the three subjects who underwent procedure 2, will be described briefly. After session 1 three different meter settings were required during the Vision-Proprioception phase. FIGURE 6 is a plot of mean performance by session. Perhaps the most striking feature is the manner in which over- and undershooting vary alike for all three values.

Beginning with session 4 the subject's task during Proprioception Only was to produce meter settings on which he had not previously worked. These were 1, 2, 3, and 4 volts. Performance for Subject 4 is plotted in FIGURE 7. Settings may be seen to be quite inaccurate during sessions 4 and 5, but in the correct order at least. Sessions 6 and 7 show substantial improvement for all except the top value. Subjects 5 and 6 also undershot this value, although not to the same extent. Questioned after the experiment, two subjects reported having been fearful of pegging the meter.

Conclusions and Implications for Further Research

The above findings are offered as exploratory, to be confirmed and extended at the first opportunity. Sketchy as they are, it is believed that they demonstrate proprioception to be a modality of such astonishing sensitivity as perhaps few have heretofore suspected. Also, the major question raised – can private stimuli be aligned to a public scale? – has, within limits, received a positive answer. The limits of training, such as durability of the subject's "calibration," transfer effects to other muscles, pattern phenomena, and idiosyncratic aspects, remain to be examined.

The deficiencies of verbal report by the subject certainly offer nothing new, but the delicacy of the task and the subject's measurable response to subtle variables suggest that the situation is one exceptionally favorable for the study of variables that determine the rise to threshold strength of those verbal responses that constitute, in Skinner's[12] terminology, "tacting one's own behavior."

A possible next step will be to examine the stability of proprioceptive control in the face of incompatible exteroceptive stimulation. For instance, if, during Proprioception Only, the well-trained subject is supposed to maintain a specific meter reading, what will be the effect of having the meter dial before him show a discrepant value? Will he drift

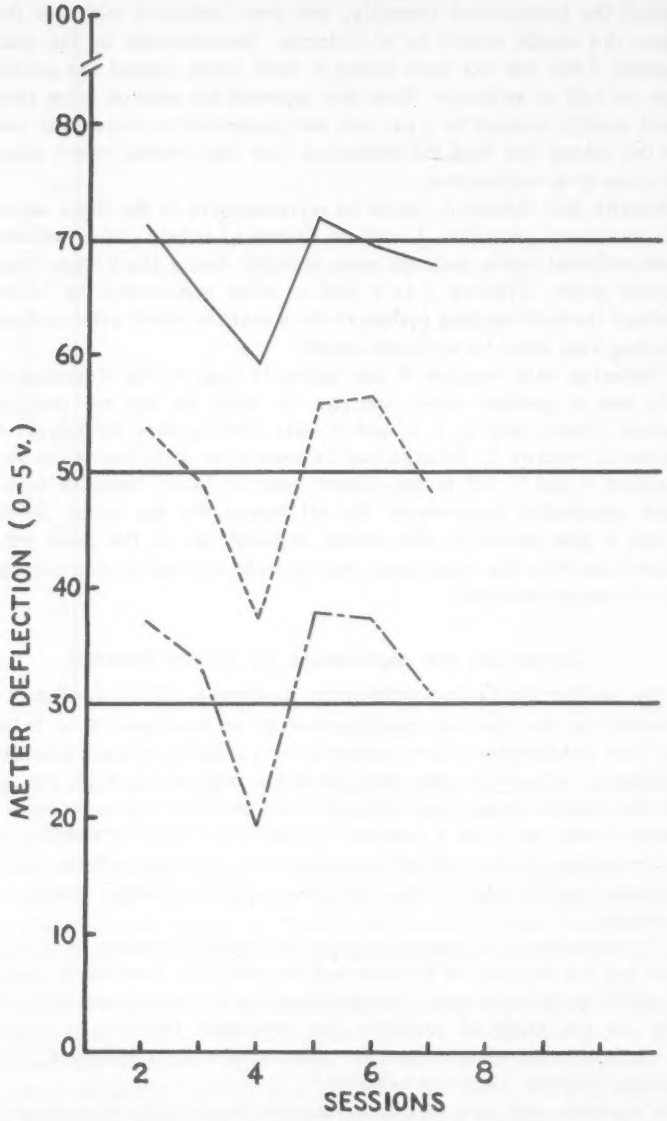

FIGURE 6, Mean values of pen-excursion midpoint by session for Subject 4, with meter setting to be approximated determined randomly by cycle from 3 values and announced just before start of Vision-Proprioception. Straight horizontal lines at 30, 50, and 70 (1.5, 2.5, and 3.5 v.) represent perfect performance; accompanying plotted lines represent subject's actual performance.

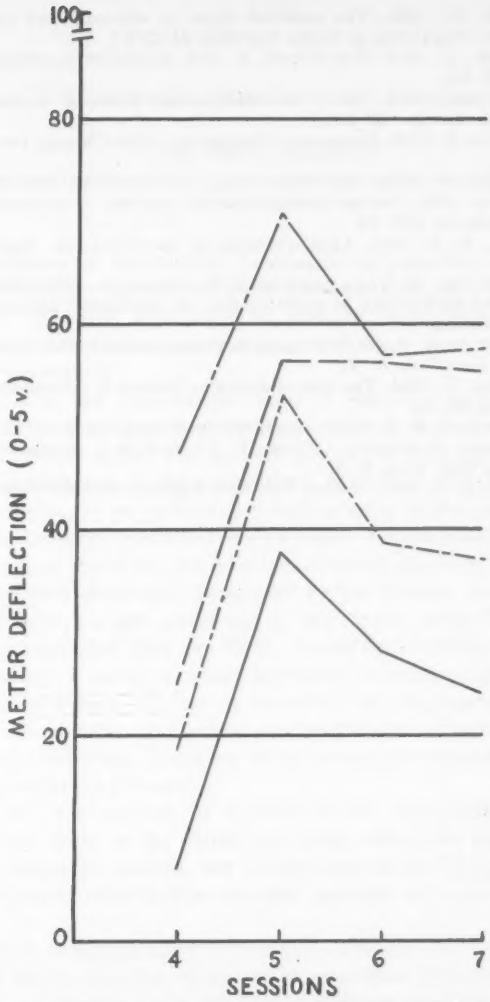

FIGURE 7. Mean values of pen-excursion midpoint by session for Subject 4, with meter setting to be approximated determined randomly by cycle from 4 values and announced just before start of Proprioception Only. Straight horizontal lines at 20, 40, 60, and 80 (1, 2, 3, and 4 v.) represent perfect performance; accompanying plotted lines represent subject's actual performance.

toward it, overcompensate, or be unaffected? Or if the meter, independently of his action, presents a moving sequence of needle positions, will the subject, on the basis of his training, involuntarily "imitate" the meter with his muscle?

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SECTION OF BIOLOGY AND DIVISION OF MYCOLOGY

I. BIOCENOLOGIC PROBLEMS IN IMMUNOLOGY*

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The problems of biocenology in relation to infection, immunity, and infectious diseases are not sufficiently known, especially by the medical profession. It seems appropriate to discuss immunology from a broad biological point of view especially as this field eventually will have practical importance.

Unfortunately the nomenclature used by various authors in this field of study is frequently very confusing, as many authors use the same terms in different ways. The primary purpose of this review will be to establish definitions of the terms used. Definitions have been chosen (or created) that, in my opinion, are best adapted to the logical development of the concepts presented in this paper. A short historical introduction to the most important problems involved will be given.

The term biocenosis was introduced by the German zoologist August Ferdinand Möbius eighty years ago in his classic work *Die Auster und die Austernwirtschaft* (Möbius, 1877). According to Möbius a biocenosis is a community of living creatures belonging to different species having, in the occupied area, all that is necessary for life, growth, and multiplication. The number of species and individuals is limited by the environmental conditions. Synonyms of biocenosis are cenosis, community, biotic community, and biome.

Since 1877 the concept of biocenosis has developed with special stress on the unity of the animal and plant world, the quantitative aspects of component species, the evolutionary aspect of its development, and the different relationships between species and individuals in biocenosis.

Pathogenic microorganisms as members of biocenotic communities are discussed chiefly from the viewpoint of parasitism. A biological concept of infectious disease as a case of parasitism was developed in the Lowell lectures in 1909 and in the Herter lectures in 1916 by Theobald Smith. These lectures were never published. In 1926 Charles Nicolle discussed these problems in his book on the origin, existence, and cessation of infectious diseases (Nicolle, 1926). In 1931 Ludwik Hirsz-

*This paper, illustrated with slides, was the first of two papers presented at a Special Meeting of the Section of Biology and the Division of Mycology on May 22, 1958.

†On a Rockefeller Foundation Travel Grant in the United States.

feld expounded his ideas about mutualism between micro- and macroorganisms as a more important biological factor than parasitism (Hirszfeld, 1931). A cornerstone in this field was Theobald Smith's book *Parasitism and Disease* (Smith, 1934). In the same year 1934 there appeared the monograph of Eugen Pavlovski, *Organism as the Habitat of Life*, in which a strictly biocenologic point of view was introduced in relation to infection and infectious diseases (Pavlovski, 1934). In 1936 Martini gave a biocenologic presentation of epidemiology and, in 1937, Hörnig described infectious human diseases in biocenologic terms (Martini, 1936; Hörnig, 1937). Problems of parasitism, ecologic, and biocenologic problems in infectious diseases were later discussed by Swellengrebel (1939), Burnet (1940, 1945), Rivers (1947), Hirszfeld (1948), Francis (1948), Allee *et al.* (1950), Makower (1953, 1956), Cameron (1956), and others.

A very important concept in the science of infectious diseases and the science of their spread is that of population as the term is used in this field. There are several definitions of this term. We shall use the relatively simple one of Rabotnov (1950), who considers all individuals of one species belonging to a biocenosis as a population. Such populations may be very great or they may consist of a very small number of individuals.

The size and number of species and individuals forming a cenosis may also be very variable. The smaller cenoses may be parts of greater ones as, for example, a leaf, a tree, a forest. Every animal or plant may be regarded as a biocenosis, consisting of one macroorganism and of a variety of populations, such as external or internal greater parasites and protozoal, fungal, or other microbial populations. Such an idea was proposed in 1908 by Dahl (Dahl, 1908).

Every macroorganism is a host to all these living creatures, living on or within it. The latter may be called *guests*. The members of a biocenosis are linked together by biocenotic relationships the most important of which are harmful or useful. If these relationships are intricate and involve two species, the members form special associations that were named symbioses by de Bary (de Bary, 1879). Unfortunately this word came to be used predominantly for mutually useful associations, so that it is better to avoid confusion by not using it at all. A better term for such associations is cooperative mutualism or mutualism.

The action of organisms of a biocenosis on other organisms is coaction (Clements and Shelford, 1939). Coaction concerning two species may cause mutual damage or destruction (disoperation) and may be useful to one and harmful to the other organism (exploitation); coaction may exist without causing damage to either side (tolerance) and it may be mutually useful (mutualism). We are concerned here chiefly with: (1) parasitism as a kind of exploitation; (2) commensalism as a kind of tolerance; and (3) mutualism.

There are many definitions of parasitism (reviewed by Dogel, 1947, Makower, 1956). I shall use the relatively simple definition of Beklemi-

shev (1945), who regards the host's body as the environment in which the parasite lives and the parasite as an organism that takes food from and damages the host.

Microorganisms causing disease damage the host and, according to definition, are parasites. As previously stated, microbial parasites are guests in the host's body, but if such guest organisms are not harmful to their host there is tolerance, and the microorganisms are then commensalic. The concept of commensalism was introduced by van Beneden (1876).

Useful microorganisms living in or on a host, both deriving profit from such coaction, are mutualistic. The relations between hosts and their microbial guests are indicated in FIGURE 1.

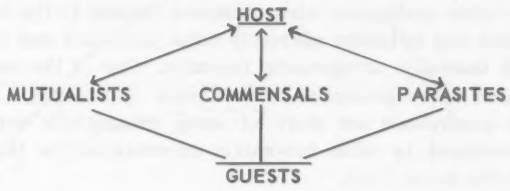

FIGURE 1

Microorganisms may live on the surfaces of the host's body (even if the surfaces have been invaginated in the course of the ontogenic development and actually are inside the body: for example, in the mucous membranes of the alimentary tract). Such microorganisms are epibiontic. If they are really inside the body – in the tissues or cells – they are then endobiontic.

A population of microorganisms from the outside world (or a epibiontic population from the body surfaces) can traverse the normal barriers and penetrate inside the body, into the tissues and (or) cells. The population of the first kind is called a transmissive population; of the second kind, a transgressive population (Makower, 1953). The establishment of a microbial population within a macroorganism is called infection.

If infection is associated with sufficient damage to the organism to cause signs and symptoms of disease, an infectious disease has resulted. As a reaction to the life of microbes inside the host's body, processes and mechanisms develop that tend to inhibit or eventually to destroy the invaders. These processes and mechanisms are immunological processes and mechanisms. The state of defense, which fights infection (either established or in course of development) is called immunity. The science dealing with infection and immunity is known as immunology.

If infection is not followed by infectious disease there is inapparent, asymptomatic, or silent infection. As long as the infection lasts the

macroorganism is a carrier. Also, during and after convalescence the carrier state may be present for a shorter or a longer period, sometimes even for life.

If asymptomatic infection is associated with immunity to other populations of the same germs or even to populations of other microbes, the infecting microorganisms may be mutualistic. This type of immunity was named symbiotic immunity by Hirszfeld (1931). If the balance between a carrier and guest microbes that are potentially pathogenic is disturbed, either a new outbreak of disease can develop (a relapse or a recurrence), or the carried microbes can be destroyed and the carrier state comes to an end.

However, at times the normal or even the pathogenic microorganisms may have not only an injurious effect on the host but also antagonistic activities on other pathogenic microorganisms present in the body. Such microorganisms may influence adversely these pathogens and may create some sort of immunity: antagonistic immunity. One of the mechanisms for such action is the production of antibiotic substances. Antibiotic therapy and prophylaxis are ways of using antagonistic activities of microbes developed in other biocenotic communities, in the infected animal, or in the human body.

A special kind of infectious process is engendered by certain disturbances in the animal or in the human body. An equilibrium is sometimes maintained by the antagonistic action of some populations of the microbes on other such populations, and when, for example, some bacterial populations are eliminated as a result of antibiotic therapy, this may lead to the spread of fungi normally held in check by the bacteria. Such complications of antibiotic therapy, frequently occuring after the use of broad-spectrum antibiotics constitute a problem of serious medical importance. Moniliasis is the most prominent of the diseases in question. Because of the mechanism of their development, these maladies can be named "desantagonistic" infections and desantagonistic diseases (Makower, 1956).

Biocenotic Relationships Between Microorganisms and Their Hosts

In every infection the number of microorganisms (or the size of the microbial population) is continuously changing. We may present this graphically as a curve in which the number of microorganisms is related to time (FIGURE 2, Makower, 1953).

A transmissive or transgressive microbial population (P_{tr}) starts to multiply after a lag period. As long as there are too few microorganisms to cause pathogenic changes associated with signs and symptoms of disease there is, of course, no disease. This period is called the incubation period. The microbes are then commensalic (P_c). When they cross the L_p line (limit of parasitism) they are parasitic and become a parasitic

population (P_p). The period of illness then begins. The number of microbes increases for a certain time; then either the host dies (mortal infectious disease) or, after a period of time, the immunologic mechanisms of the macroorganism, eventually helped by therapeutic measures, cause a decrease in the number of living microbes. When the L_p line is again reached the period of convalescence begins. The parasitic population again becomes commensalic ($P_p \longrightarrow P_c$) or even mutualistic ($P_c \longrightarrow P_m$). This last possibility is realized when the persistence of the microbic population in the body is connected with immunity (symbiotic or infectious).

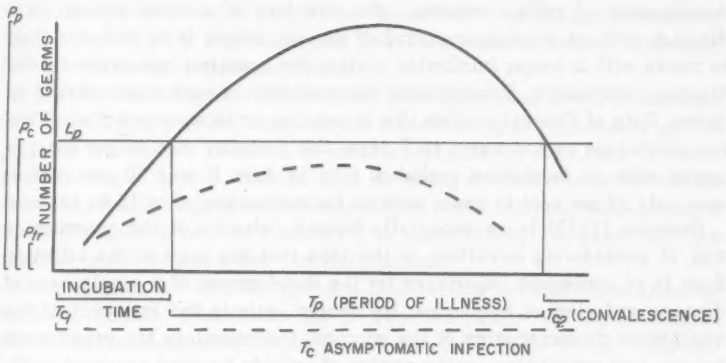

FIGURE 2

If the infecting population does not grow quickly enough before the internal or external antimicrobic forces come into action, the microbial population will not reach the L_p line and the whole infectious process will be equivalent to an extended incubation period (broken line, FIGURE 2). Instead of an infectious disease, a subclinical, inapparent, asyptomatic, or silent infection will be the result. The microbial population will be commensalic or mutualistic as in the previously mentioned carrier state. Indeed, a carrier state is present here, but without a previous infectious disease.

The above discussions are relevant to a relatively short and intensive, acute infectious disease, but they apply also to slowly developing diseases of long duration, that is, chronic infectious diseases. Often, as in tuberculosis or syphilis, a chronic infectious disease prevents further infections of the same kind; here we have another kind of symbiotic immunity – intrainfectious immunity (Sergent).

The outcome of the encounter between macroorganism and microorganisms depends to a great degree upon the size of the infectious dose, as described by Theobald Smith (1934), Webster (1946), Makower (1952,

Congress of Polish Microbiologists, 1953; 1956), Bormann (1953), and others. The length of the incubation period may vary directly with the number of infecting microbes. In a smaller P_{tr} there must be a greater number of generations to reach the L_p line; so the incubation period must be longer. This is seen in the experiments of Contreras and Melnick (1953), in which newborn mice were infected with small or large doses of Coxsackie viruses. The incubation period (from infection to the development of paralysis) was approximately twice as long after small doses of virus as after large doses.

In the course of a long incubation period there is more time to develop sufficient defense mechanisms, as was done by Pasteur to prevent the development of rabies infection after the bite of a rabid animal. In a disease with an incubation period of varying length it is probable that, in cases with a longer incubation period, the organism can overcome the disease more easily. Consequently the mortality in such cases should be lower. Data of Kümmel confirm this in relation to tetanus infection. When the incubation period was 1 to 7 days, the mortality was 90 per cent; in cases with an incubation period of 6 to 14 days it was 50 per cent; it was only 32 per cent in cases with an incubation period of 15 to 42 days.

Bormann (1953) is an especially fervent defender of the quantitative way of considering infection, of the idea that the size of the infecting dose is of paramount importance for the development of a single case of disease and also of epidemics. He denies entirely the influence of the qualitative characteristics of the microbe. Unfortunately his experiments are not very convincing, as the number of animals he used was very small.

In my opinion not only quantitative changes, as described in FIGURE 2, but also qualitative changes in the host influence the course of infection and of the infectious disease. Microbial changes of this sort may be either mutations or modifications. The changed strains resulting from genetic or environmental variation may eventually multiply faster or slower (showing a connection between qualitative and quantitative changes). The tropism to certain tissues may be altered (for example, in neurotropic strains of influenza or yellow fever viruses). Pathogenicity for certain hosts may be changed as, for example, in the classic experiments of Pasteur with street and fixed rabies virus. New antigenic patterns may develop; these are often seen in influenza viruses. Serial passages in animals are particularly prone to be associated with changes in pathogenicity for these or other animals, chiefly in the direction of an increase of pathogenicity if the animals are susceptible to the microbial infection.

The question of the development of special highly pathogenic strains during epidemics (epidemic strains) is still a matter of controversy. In light of the newest investigations, however, it cannot be denied that the development of such strains is probable.

A classic example of the state of pathogenicity, depending upon the effect of external conditions on the host, was furnished by Pasteur in his

experiments with the immersion of birds in cold water. In this way the birds became sensitive to the anthrax bacillus, which does not grow at the normal body temperature of birds.

A new mechanism for inducing pathogenic properties was discovered in diphtheria bacilli. Nontoxic strains could be converted into highly toxic ones by infecting the bacilli with an appropriate bacteriophage (Freeman and Morse, 1953). Thus hyperparasitism enhances parasitism; a new biocenotic relationship changes the effect of microbes on their host.

The immunologic mechanisms that inhibit or destroy the infecting microbes are mostly cellular (phagocytosis, inflammation – Elie Metchnikoff), humoral (antibody production – Emil von Behring, Paul Ehrlich) or metabolic (René Jules Dubos). The role of the immunologic forces that can be immediately applied at the portal of infection is very important (effective immunity, Makower, 1950).

Symbiotic immunity in carriers has already been discussed. It has been established that such immunity exists in salivary gland disease in guinea pigs, in psittacosis in birds, in choriomeningitis in mice, and in infectious anemia in horses. In smallpox, measles, and some other infectious diseases, the lasting immunity is probably not connected with a carrier state. The cause of this immunity has been widely discussed and the problem cannot be considered entirely solved.

Some clue to the development of a long-lasting immunity was given by MacLeod (1953), who connected it with the length of the incubation period. If the incubation period is sufficiently long to permit, after another encounter with the microbes, a sufficiently strong rise of immunity, especially of the antibody response, then the microbial invaders will be inhibited and the disease will not develop. A new infection will have a booster effect; in a sense it will work as a natural vaccination. The new infecting population can then be considered as mutualistic, as it keeps high the immunity level. This mechanism probably exists in diphtheria and scarlet fever. Sometimes the spread of the microorganisms, which are infectious but not disease-producing, is very large and we then have some sort of subclinical epidemics, which eventually cause natural vaccination of human or animal populations on a big scale (for example, in poliomyelitis). When the incubation period is short, as in influenza, this mechanism cannot operate and the duration of the immunity is short.

Even in diseases with long incubation periods such "second encounters" do not always produce such happy results. If the infecting dose is very great, the strain very pathogenic, and the host in a bad immunologic condition, there may be a second course of the infectious disease, as seen sometimes in the above-mentioned and other diseases.

A special kind of immunity, described by Ehrlich, is atreptic immunity. It depends upon the lack in the host of some nutrients essential for the parasite. The natural immunity of many animal species is based on this mechanism. If another microbial population supplies the lacking nutrients,

it allows the multiplication of the first one. In such a way protreptic sensitivity (Makower, 1956) may be created as a result of mutualistic or synergistic action between two microbial populations.

One microbial population in the host can stimulate another such population. This may be caused by the secretion of metabolic products (toxins), by diminishing the immunity of the host (for example by creating a state of anergy in influenza or measles), or by hyperparasitism, as in diphtheria bacilli infected with bacteriophages.

We have discussed the problem of infection and immunity in a biological system consisting of one host and one or more microbial populations living within the host. The association may be quite complicated, the pathogenic agents forming links between several animal (or animal and plant) species. In such a way, especially with the help of arthropod or other vectors, biocenotic connections of a more or less complicated nature may develop. Several examples of two- or multimember biocenotic relationships of this kind are given in TABLE 1 (Makower, 1953).

Biocenotic Interrelationships Between Microorganisms and Cells

The biocenologic coactions between microbes and their hosts may exist on different levels. We have discussed the interrelationships that occur in one infected host. The next higher level is the common life of microbes with animal or human populations, eventually belonging to two or more species. The lower level is concerned with coactions between parasitic (or commensalic) microbes and cells of metazoan or protozoan organisms. This question, which was the subject of an interesting symposium in Galveston, Texas, two years ago (*Host-Parasite Relationships in Cells*, 1957), will be discussed briefly.

The theory of phagocytosis was the first scientifically based theory of immunity. Metchnikoff linked the ingestion and digestion of microbes by the phagocytic cells with intracellular nutrition, as seen in protozoa, sponges, and some other metazoa. Evolution of the alimentary tract and of extracellular digestion rendered the digestive role of phagocytes unnecessary. However, these phagocytes did not perish in the course of evolution and remained as scavenger cells with very important defensive functions, which were accelerated and increased in higher animals by the presence of antibodies. A phagocyte containing ingested microbes is a biological system analogous to an amoeba with microorganisms ingested as food. This last case is an example of an exploitative coaction, a predatory system as in the case of the phagocyte with ingested microbes. Sometimes the predator-prey relation may be reversed, as when microbes multiply in the protozoan host or in the phagocytic metazoan cell; they may damage these cells and so become parasitic.

In the metazoan host there is generally a population of microorganisms and some sort of a predatory phagocytic population (or such different

TABLE 1

TABLE 1

TWO- AND MULTIMEMBER BIOCENOSES

Number of species			*Examples of Cenoses*			*The microbial parasite*
Members of cenosis	*Hosts*	*Microbial parasites*	*Disease*	*The host*		
				Principal	*Intermediate*	
2	1	1	Typhoid fever	Man	–	*S. typhosa*
			Gonorrhea	Man	–	*N. gonorrhea*
			Syphilis	Man	–	*Tr. pallidum*
			Smallpox	Man	–	Smallpox virus
3	2	1	Malaria	Man	Anopheles	*Plasmodium*
			Relapsing fever	Man	Louse	*Sp. recurrentis*
			Relapsing fever	Man	Tick	*Sp. duttoni*
			Typhus fever	Man	Louse	*R. prowasekii*
4	3	1	Rickettsial pox	Man, mouse	Mite	*R. akari*
Numerous	Numerous	1	Anthrax	Domestic animals and man	–	*B. anthracis*
			Q fever	Man, domestic, wild animals	Ticks	*C. burneti*
3	1	2	Vincent's angina and related diseases	Man	–	*Sp. vincenti* *F. fusiforms*
5(6)	3(4)	2	Swine influenza	Swine	Swine lung worms Earthworms	Swine influenza virus *H. influenza suis*
Numerous	Numerous	Numerous	Gas gangrene	Man, animals	–	Many species of the genus *Clostridium*

phagocytic cells as granulocytes, monocytes, macrophages, reticuloendothelial cells, and even different phagocytic populations). When the phagocytic action is strong the free-living microbial populations are quickly ingested. The outcome of the encounter between phagocytes and microbes depends upon the ability of the microbes to resist this phagocytic action. In the course of evolution several microbes succeeded in adapting themselves to an intracellular life in both phagocytic and other cells. On the other hand, the disintegration of microbes in the phagocytes liberates the antigenic substances and is perhaps a step in the evolution of humoral immunity.

The microorganisms that adapted to intracellular life, such as viruses, for the most part behave in cells like true parasites. Their immediate environment is the cytoplasm or nucleoplasm, and it is only while they are being transported from cell to cell and during viremia that they are under the influence of tissue fluids and blood. The presence or absence of antibodies in this environment may decide the outcome of infection.

The presence in the cell of certain eventually nonpathogenic or only slightly pathogenic virus populations may cause immunity to other strains, even if the latter are very pathogenic. The mechanism of this interference is intracellular. It is not well understood as yet. Interference is a kind of cellular immunity based on antagonistic tendencies between more or less similar viral strains, perhaps due to competition for common metabolities. The interfering virus may act as a mutualist to its immediate host, the cell, and also to the host on a higher level, that is, to the macroorganism. Interference is especially valuable when it can prevent the development of an infectious disease until a solid humoral immunity can be produced.

Lysogenic bacteria furnish another example of immunity caused by intracellular nonpathogenic viruses (Lwoff, 1953). The so-called prophage is fixed in the genetic mechanism of the bacterial cell and there it prevents pathogenic phages from destroying its host. The problem concerning the nature of the prophage, which is a "captured" infective agent or a special cell constituent, is of great theoretical importance.

An especially interesting but insufficiently understood kind of coaction between viruses and cells is performed by the so-called masked viruses (Shope, 1942, 1950). These viruses not only live in cells – without doing them any harm – but cannot ordinarily be recovered from cells in an infective form. Apparently their multiplication, if any, is very slow.

The action of certain viruses on cells may lead to the rapid multiplication of the latter, and in this way they may be the cause of tumors. These viruses do not damage the cells grossly, but they continue to behave as parasites because they ultimately may cause the death of the cell and of the whole organism.

A special artificial association of cells with viruses (and with some other microorganisms) has been the object of detailed studies in the last few years as a result of the development of tissue culture techniques

in virology. Infected tissue cultures may be regarded as cenoses *sui generis*, whereas pure cultures of bacteria in artificial media may be considered as populations. Most viruses behave in tissue cultures as strict parasites, exerting cytopathogenic effects.

While viruses, rickettsiae, and some bacteria are adapted to intracellular life mostly in a parasitic manner, there are some microorganisms that live in cells as true mutualists. These are the so-called symbionts of insects. There are two kinds of symbionts: those living extracellularly and those living intracellularly. The latter symbionts have developed from the former. The intracellular symbionts are chiefly located in special organs: mycetoms or bacterioms.

The first mycetom was described in 1850 by Franz Leydig in aphids. Bacteria in mycetoms were found during the years 1886 to 1888 by Blochmann (quoted by Steinhaus, 1949), and their useful role was detected by Krassilschik, a pupil of Metchnikoff, in 1889 and 1890. Since then much has been learned concerning these symbionts, especially from Buchner (1956), who devoted his life to studying and describing them in minute detail.

In addition to bacteria and fungi, other microorganisms were found functioning as insect symbionts, for example the rickettsiae. Most symbionts cannot be grown on artificial media. Also, insects cannot live without their symbionts.

The intracellular symbionts are transferred in many species to the next generation by the transovarial route. Occasionally very complicated arrangements facilitate entry of the extracellular symbionts into ova or larvae.

The embryonic development of mycetoms as organs that are specifically brought into existence for the housing of symbionts has been used in investigating the evolution of some insects and the relations existing between different groups of these animals. The evolution from extracellular to intracellular symbionts obviously developed through the close association of extracellular symbionts in the alimentary canal with the surfaces of the cells that line the alimentary tract. The symbionts then entered these cells, which moved by invagination outside of the intestine. The cells finally separated from the intestine and developed into special independent organs (Steinhaus, 1949).

From generation to generation new hosts are infected by the symbionts, but this infection is mutually useful. There must be some sort of immunity in the host that strictly limits the level of multiplication of the symbionts. This is probably also true in relation to extracellular symbionts. Cellulose-digesting protozoa and bacteria are especially interesting examples of these microorganisms. Termites cannot live without the presence of flagellates in their alimentary canals. Protozoa and bacteria play an important role in digestion in ruminants and other herbivores.

Another mutualistic association between multicellular and unicellular organisms is represented by the *Zoochlorella* and *Zooxantella* (discovered by the Polish microbiologist Leon Cienkowski) living in flagellates, coelenterates, and some other animals.

Such associations are not always mutualistic. The larva of a planarian, *Convoluta roscoffiensis*, swallows green flagellates, which penetrate its tissues and multiply there (Cameron, 1956). Without this infection the worm dies. Infected mature worms do not ingest food from the environment, but they feed on their own flagellates. After laying eggs, the worm digests all its symbionts and subsequently dies from lack of food. This is indeed a strange biocenotic relationship, especially for the host. The symbiont, which is in this case a sort of prey, does not depend for its existence on its host; primarily it leads an independent life.

Biocenotic Interrelationships Between Microorganisms and Animal and Human Populations

The biocenologic associations between microbial populations and populations of higher animals or men are the subject of epidemiology in the broadest sense. Great progress in this complicated and difficult field was achieved by introducing experimental methods in epidemiological work. As a result of these methods a new branch of science, experimental epidemiology, was developed. It has a very close connection with epidemiology as a social science.

The first attempt to introduce experimental methods in epidemiology was made by Topley in 1919 (quoted in Greenwood *et al.*, 1936). British investigators from the London School of Hygiene summarized their results in monographic form in 1936 *(idem.)* and in the textbook on bacteriology written by Topley *et al.* (1955). Next in importance was the work of investigators in the United States, headed by L. T. Webster of the Rockefeller Institute for Medical Research, New York, N. Y.

The most important problems of experimental epidemiology are the dynamics of the spread of infectious diseases and of infecting microbes in artificially maintained populations of experimental animals, chiefly mice; the development of immunity and its influence on the epidemiologic pattern; the statistical analysis of cases of disease and of deaths; and an evaluation of the influence of environment.

The principal method used in this work consists in introducing newly infected individuals into the population (or into the herd, as Topley put it), in which the infection spreads in natural ways. Also, new uninfected mice are introduced. According to British workers immunity in mice increases with the time they spend in the cage. A great majority of the mice is infected in a short time after arrival. The high initial mortality decreases with time, and some sort of equilibrium develops between microbes and hosts. However, this balance is not stable and, under the influence of environmental factors, a new epidemic may ensue. The size

of the infectious dose is important, but it is not the only factor on which the result of the encounter depends. The state of acquired immunity is another important factor, as are also some chance factors. According to the British scientists, genetic factors play a smaller role.

Quite a contrary opinion is held by Webster (1946) and others of the United States school. Webster describes two types of epidemics: one having no previous association between microbes and hosts, and another having a long association between them. By special methods of selection Webster obtained strains of genetically pure mice with greater or smaller immunity. In a mixed population more sensitive individuals are chiefly the victims of infection.

There are discrepancies between the results of the London and the new schools of experimental epidemiology. Future work will certainly solve them. The influence of different factors on the spread of infection, as surmised from experiments of both schools, may be eclectically summarized as depending on four kinds of variation: (1) variation of the infective dose; (2) qualitative differences in the microbial population; (3) congenital differences of immunity in particular individuals of the mammalian population; and (4) differences in acquired immunity.

A trend quite opposite to those in experimental epidemiology is represented by field investigations conducted by many workers. These investigations were originated by Theobald Smith's discovery that Texas fever in cattle is transmitted by ticks. This finding and the elucidation of the transmission of malaria by Sir Ronald Ross and Giovanni B. Grassi were milestones in a very fruitful field dealing with vectors of infectious diseases. It was soon found that many pathogenic microorganisms transmitted by arthropod vectors have natural reservoirs in animal populations.

Especially valuable were investigations made in the Rocky Mountain Laboratory, United States Public Health Service, on Rocky Mountain spotted fever (Parker, 1930). Ticks as vectors of this disease were thoroughly investigated, endemic foci of infection were described, conditions in which men are likely to be infected were established, and methods of control were introduced, the most important being clothes that prevent ticks from attacking men and inoculation with a tick vaccine. Reservoirs of rickettsioses in mammals were also investigated.

Work done in the United States in connection with sylvatic plague and tularemia also threw much light on the spreading of plague and tularemia bacilli by wild rodents and other mammals; also on their ectoparasites and on the effect of these foci of infection in human beings.

These investigations were made on biocenologic lines but without introducing the concept of biocenosis. Such investigations may be considered as unconsciously biocenologic. A consciously biocenologic approach was introduced in this field by Eugen Pavlovski and his school in Leningrad (Pavlovski, 1951 and 1954, and Petrishtsheva, 1954), but some of the findings of this school were anticipated by workers in the United States and elsewhere.

Pavlovski's theory of natural foci of transmissive diseases was introduced in the late 1930s as a result of investigations made in Siberia on tick encephalitis (spring-summer encephalitis), its virus, and its hosts. The essence of this theory is expressed by Pavlovski in the following way: "...the pathogenic agent, its specific vector and animals (reservoirs of microbes) exist during an unlimited period of time in nature, through many generations, as a part of many biocenoses, independently of man, and that in the course of the bygone evolution, as also in the present time."

Diseases of this kind are called transmissive diseases by Pavlovski. They are characterized by the following features: (1) the germs exist and circulate in nature independently of man, in old biocenoses consisting of vertebrate animals and of bloodsucking invertebrate parasites; (2) more or less intense polyphagy of the vectors makes these biocenoses more or less complicated; (3) infected ectoparasites, by biting man, include him in the long-established biocenoses as a new member; (4) there exists a tendency of "domestication" in such diseases under certain conditions, and the diseases may spread in villages and towns; and (5) these diseases occur in special geographical sites such as deserts, steppes, forests, and mountains at certain seasons of the year.

To such diseases belong, for instance, tick rickettsioses and spirochetoses, seasonal encephalitides (tick- and mosquito-borne), leishmanioses, tularemia, Q fever, and yellow fever.

Wild animals are donors and acceptors of infectious germs. Man is primarily an acceptor; he rarely infects the arthropod vector. The natural focus of a transmissive disease is a territory of a definite geographical site in which the infectious agent has sufficient opportunities to circulate continuously from the donor through a vector to an acceptor.

The natural foci may be very small (for example, dens of rodents) or large and confluent (for example, forests). On the borders of the different geographical sites arise especially interesting biocenotic relationships (Petrishtsheva, 1954). Here the fauna of vertebrates and their ectoparasites are generally richer. The interchange of parasites is more rapid. For this reason, in such areas possibilities for the establishment of diseases from natural foci are very great.

Bacteremia or viremia in mammals and birds is usually of short duration, as these animals develop humoral immunity. In the Siberian taiga about 50 to 80 per cent of the wild animals in a natural focus have antibodies to the virus of tick encephalitis; the number of animals harboring virus is much smaller. Arthropods that harbor the virus for a much longer period may be regarded as true reservoirs of infection.

Due to the language barrier the work done in Siberia remained unknown in the United States and other western countries in which work on similar lines was also done. As examples of this work I shall discuss briefly researches done on Q fever and rickettsial pox and the recent investigations made in Utah on several other diseases.

After the discovery of the *Rickettsia* that causes Q fever, Burnet and other Australian workers made extensive investigations of Australian arthropods and vertebrates in which *Rickettsia* circulate in nature (Burnet, 1942; Smith, 1940, 1941; Derrick and Smith, 1940). In California similar investigations in domestic and wild animals were undertaken by Huebner (Huebner *et al.*, 1949-1951; Huebner, 1950), who elucidated the complex ways in which *Coxiella burneti* passes through natural reservoirs. Huebner performed analogous work in relation to another *Rickettsia* that he discovered in a very rare disease, the rickettsial pox (Huebner, 1950).

Investigations on lines similar to those performed by Soviet workers were started at the Dugway Proving Ground in Utah under the direction of Woodbury *(Symposium on Ecology of Disease Transmission, 1957).* The project, sponsored by military and civil agencies, was begun in December 1952; its cost was 3 to 5 million dollars yearly. Woodbury (1957) describes the aim of this work "as a safety measure or an insurance program to protect the health and welfare of neighboring human populations from the ravages of disease, either introduced or endemic, that may occur in the native fauna." Woodbury (1957) regards this work as "a new approach to the study of disease dissemination by evaluation of the impact of ecological conditions upon disease in the native fauna." Essentially the same ideas were expressed by the United States and the Soviet workers already quoted.

The great importance of investigations of native fauna in relation to diseases of man and domestic animals must be emphasized. Such investigations must be done on a world-wide scale; eventually they should be coordinated and organized by the World Health Organization of the United Nations.

Phylogenesis of Infectious Diseases and of Immunity

The history of coactions between macro- and microorganisms eventually resulting in disease could throw much light on many problems in this field that are still poorly understood. Unfortunately paleobiology is of little help in this respect, and we are obliged to draw conclusions from findings made by comparative methods. The first attempt of this kind was made by Metchnikoff in his *Lectures on Comparative Pathology of Inflammation* (1892). In this publication he gave a systematic survey of the development of pathological and defensive reactions throughout the animal kingdom. He started with protozoa infected by small unicellular fungi.

Metchnikoff emphasized the importance of cellular reactions in defensive mechanisms. In his opinion the evolution of immunity was identical with that of cellular immunity. Originally Metchnikoff considered infection and its conquest as a question of nutrition, the struggle between

hosts and their microbial parasites ending in either of two ways: the parasites ate their host or the host consumed the parasites.

I have already mentioned the analogy between the ingestion of smaller microorganisms, as food, by protozoa and the ingestion of bacteria by phagocytes. A contrary situation occurs when protozoa swallow microorganisms that become parasites, as when the flagellate *Euglena* swallows the fungus *Chytridium*. The same thing occurs in the ingestion of staphylococci by phagocytes.

The first systematic effort to understand how infectious diseases originate, how they develop, and how they eventually cease to exist was made by Charles Nicolle (1926), who thought that putrefactive bacteria, which develop from saprophytic species and are able to live only in necrotic and dead tissues, were the first organisms to have approached the road to parasitic life. These early invaders were able (and still are able) to live not only in necrotic tissues but also, to a certain degree, in living tissues, as do the bacilli of gas gangrene. They live freely in nature and find their way to the interior of higher organisms by way of a trauma, or by chance, without having special mechanisms enabling them to form new infecting chains. Diseases caused by them are basically not contagious, although they may be severe and may cause great mortality.

The next step was the study of the development of true bacterial pathogens, for example typhoid, plague, or tubercle bacilli. These organisms have a moderate capacity for living in free nature.

Some bacteria, rickettsiae, and viruses are totally devoid of the capacity for living outside living cells.

The first contacts between microorganisms and their potential hosts may lead to very severe and often mortal disease. Long associations between germs and their hosts can lead to mutual adaptations, to commensalism, and to mutualism. This last concept was broadly developed by Ludwik Hirszfeld (1931, 1948), who thought that absolute pathogenicity, depriving the microbes of the capacity for living in dead tissue subjects them to the action of antagonistic, defensive mechanisms developed under the impact of infection in the host. Such organisms have a smaller opportunity to survive in the struggle for existence than more peaceful microbes. Tolerance is biologically more useful than extreme parasitism or, as Hirszfeld states: "Symbiosis is a more rational and not only ethically higher principle than parasitism, and accommodation, or adaptation to the stimulus, has the same right for existence as the destruction of the carrier or the harmful stimulus. Pathogenicity can be understood only as an intermediate link in the transition to symbiosis."

As a result of long association, mutual adaptations cause an endemic (not epidemic) spread in the partly immune autochtonous populations, including those of some carriers. The same microbes in a new population, without previous epidemic experience, can cause severe epidemics.

Infectious diseases can originate as a result of the adaptation of commensalic microbes to parasitic life. They may develop into new commensalic or mutualistic associations and also into parasitic ones, proceeding in cycles from commensalism to parasitism, and sometimes even to mutualism (Makower, 1953, 1956). The coactions between microbes and higher animals can often develop in different directions.

These are general reflections. In some cases the origin and development of infectious diseases is more specifically known. Transmissive diseases with natural foci of infection present examples of such cases. Here, potentially pathogenic microorganisms are acquired by man in nature, and the principal cause of disease is preadaptation of the "wild" microorganisms to the human organism.

In typhus fever, rickettsiae, which are primarily parasites and commensals of the arthropods with which they exist in an old and usually peaceful association, long ago infected rats and other rodents. In both groups they now behave chiefly as commensals. Man is a relatively new host, and so is his ectoparasite, the louse. Both species react very severely to infection with the typhus fever rickettsiae, which are even more deadly to the louse than to man.

Many protozoa adopted a parasitic form of life involving hosts of several species, sometimes linked by their parasites in very complicated biocenoses. For example, trypanosomes lived primarily in lower animals, chiefly insects, from which state they adapted themselves to parasitic and later to commensalic life in mammals (Hoare, 1943). *Trypanosoma brucei,* a parasite of antelopes, was probably the parental form of some other animal trypanosomes and also of the extremely pathogenic human trypanosomes, *Tr. gambiense* and *Tr. rodeniense,* infective agents of sleeping sickness that have a potent vector in the tsetse flies.

A very interesting theory of the origin of viruses was developed by Green (1935), Laidlow (1938), and Burnet (1945). These investigators consider viruses to be descendants of more developed microbial forms that have originated by retrograde evolution caused by their extreme intracellular parasitic life. In this way they lost most of their previous morphologic and physiologic characters. This possibility cannot be dismissed but, on the other hand, we know that very old associations of the same kind have not caused insect symbionts to become viruses. The theory of the development of viruses (or of some viruses) from specific constituents of the cell cannot be completely dismissed.

The Importance of the Biocenologic Approach and its Future

I conclude my discussion of the biocenologic problems of infection and immunity by suggesting that this field of study may have not only theoretical but also some practical applications. Theobald Smith once said: "The objectives of research as a mere accumulation of data or the display and parading of acquired knowledge in a world otherwise in motion

is outmoded. Penetration into mysteries may thrill the penetrator and occasionally others, yet this is not enough. Discoveries and inventions must be made to yield some contribution toward that rather vague goal, the welfare of mankind."

Today the most important practical achievements of the biocenologic approach to infectious diseases consists, in my opinion, of some of the results of experimental epidemiology, especially concerning the role of genetic factors and of vaccinations, and results of the investigations of the Soviet and the United States workers concerning diseases having natural foci. The knowledge of biocenology can add new weapons in the fight against infectious disease. In furtherance of this aim it will be necessary (1) to influence the humoral and the cellular environment of the parasites inside the animal body (through such factors as *p*H, hormones, nutritional adjustment, and vaccination); (2) to influence the parasitic population by introducing their microbial antagonists (some earlier attempts in this direction, including Metchnikoff's, have not succeeded); (3) to influence microbes that may cause disease as a result of the desantagonistic activity of antibiotics, by appropriate antifungal and microbial therapy (on microbes that have become antibiotic-resistant and have established normal microcenotic relationships); (4) to influence the establishment of solid immunity in patients treated with antibiotics by simultaneous or subsequent immunization with killed or live vaccines consisting of the appropriate microbes (as prophylaxis against relapses and recurrences in typhoid and typhus fevers); and (5) to fight some animal pests by establishing new biocenologic relationships, as was done in the biological warfare against rabbits in Australia.

Some of these methods are under study in several laboratories, including our department of the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw. We hope that the results of this work will be ready for presentation in the near future.

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II. TULIPOMANIA AND THE CONCEPT OF DISEASE*

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The tulip was unknown in Europe until the Sixteenth Century; one of the first large tulip plantations was started from Turkish bulbs in Flanders about 1560. Soon the cultivation of the tulip became a craze. When the tulipomania reached its peak in Holland, between 1634 and 1637, a single bulb of *Semper augustus* was sold for as much as 5,500 guilders. Some of the types of tulips that achieved the greatest fame are well known from the handsome illustrations in Dutch, French, and English books of the Seventeenth, Eighteenth, and Nineteenth Centuries, as well as from contemporary paintings. There is no doubt that during the tulipomania and until recently the highest prices were paid for variegated flowers. Until a few decades ago, indeed, nonvariegated flowers were rarely illustrated in books because they were regarded as inferior.

It was early recognized that tulips raised from seed do not produce variegated flowers at first. These plants (called breeders) have petals of uniform color – they are "self-colored" as the expression goes – and their bulbs may continue to produce self-colored flowers for several years. Then, after an unpredictable period of time, there occurs a phenomenon referred to as "breaking" that results in variegation. From then on the bulbs of "broken" tulips continue to produce variegated flowers.

In 1928 tulip breaking passed from horticultural lore into virus science when D. M. Cayley in England and M. B. McKay in the United States demonstrated that the change in the appearance of the flowers was in reality the result of a viral infection of the mosaic type. Under natural conditions the virus is transmitted by aphids, but infection can also be achieved by experimental procedures.

The most striking manifestations of breaking are changes in the distribution of anthocyanins in the epidermis of the petals, which become splashed or striped with different colors. In addition, broken tulips are likely to be less vigorous than the self-colored plants and to produce fewer bulbs. In a number of cases, indeed, death of the infected plant occurs after a few generations. However, it is also true that some infected broken tulips have been in cultivation for more than 350 years. For example, the Zomerschoon tulip has been known since 1620 and probably existed in Turkey before being introduced into Europe. When propagated by bulbs, a procedure that transmits the virus from generation to generation, these tulips continue indefinitely to produce broken

*This paper, illustrated with slides, was the second of two papers presented at a Special Meeting of the Section of Biology and the Division of Mycology on May 22, 1958.

blooms, but they exhibit no evidence of cumulative weakening effect of the infection. It is almost ironical to call disease a condition that has contributed to the spread of these broken tulips all over the world.

Flower breaking caused by various types of viruses has now been recognized in several other plant species – the wallflower, the larkspur, the pansy, and the camellia. Certain other ornamental effects are also known to result from mottling of the leaves due to interference by various kinds of viruses with the production of chlorophyll. The pale-veined type of honeysuckle is probably the most recently recognized example of this type. Among the plants that became established as ornamental species because of a mosaic-type viral infection, one of the best known is *Abutilon striatum* var. *Thompsonii*, known in florist shops as flowering maple.

Virologists regard the broken tulips and the ornamental *Abutilon* as diseased plants because they are virus-infected – this despite the fact that infection does not affect the survival of these plants and has increased their world distribution. It is true that viral infections may prevent broken tulips and variegated *Abutilon* from achieving their maximum potential size, but it is also a fact that the viruses give to these plants an ecologic advantage by encouraging their spread. Size is not the most significant criterion of biological success. By making the infected specimens of tulips and *Abutilon* more interesting individuals, viruses have given them another kind of biological advantage under the proper ecologic conditions.

DIVISION OF MYCOLOGY

TOXICITY OF *STACHYBOTRYS ATRA* FOR ANIMALS*

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INTRODUCTION

A survey of European literature indicates that certain strains of a saprophytic fungus, *Stachybotrys alternans*, previously considered harmless, have caused the death of a large number of horses. Death was attributed to ingestion of various types of straw containing a toxic substance formed during growth of the fungus. Vertinskii,[1] Drobotko,[2] Drobotko *et al.*,[3] Vyshelesski,[4] and other European workers have reported in detail on various aspects of this toxicosis, which they named stachybotryotoxicosis. Gajdusek[5] in 1953 published an English review of this and other fungal toxicoses.

The toxicosis in horses described by these writers is seasonal in distribution and exists in a typical and an atypical form. The typical form that follows prolonged ingestion of small amounts of toxic fodder is divided into 3 stages. The first stage appears within 2 to 10 days and is characterized primarily by a stomatitis with subsequent necrosis, ulceration of the oral mucosa, and formation of fissures at the mucocutaneous junction of the lips. Excessive salivation and enlargement of the submaxillary lymph nodes frequently occur. The duration of the symptoms of the first stage may vary from 8 to 30 days. The second stage is characterized by a progressive thrombocytopenia, leucopenia, subsequent agranulocytosis, and increased prothrombin time. Focal necrotic lesions are frequently observed in the oral mucous membranes. The duration of this stage varies from 5 to 20 days. In the third stage the body temperature becomes elevated (to 104.9 to 106.7° F.) and a continued decrease in number of thrombocytes and leukocytes occurs, the latter frequently being as low as 100 per cc. of whole blood. In addition, a rapid, weak pulse and arrhythmia are manifest. Diarrhea and dehydration are frequently observed. Local lesions consist of a large number of necrotic areas on the mucosa of the cheeks, gums, frenum of the tongue, on the hard and soft palate, and on the lips. The third stage, which is usually fatal, extends from 1 to 6 days.

The atypical form that follows ingestion of large quantities of toxic roughage develops within 72 hours and is characterized chiefly by

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nervous dysfunctions. Complete loss of reflex response, or hypersthenia, and hyperirritability occur, followed by a progressive loss of vision. Body temperature becomes elevated (to 105.8° F.), and anorexia develops. Although the atypical form may last from 1 to 3 days, symptoms may be observed within 10 hours, followed by death within 24 hours. Most animals afflicted with the atypical form perish.

Autopsy findings in horses that die from both forms of this toxicosis consist primarily of extensive hemorrhages and necrosis in the parenchymatous tissues.

Although limited studies have been made in the United States in recent years regarding the role of various fungi in mycotoxicoses in small and large animals, the significance of *Stachybotrys* has not been included. The present studies were made to determine whether various strains of *Stachybotrys atra** readily available in the United States, when cultured on various substrates, would produce substances toxic not only to horses but to other animals as well.

MATERIALS, METHODS, AND RESULTS

Screening of strains for toxicity

According to Vertinskii,[1] Vyshelesski,[4] and others, there exist toxic and nontoxic strains of *S. atra* that can be differentiated on the basis of dermal toxicity. Ether extracts of straw on which toxic strains had been cultured, when applied topically on a shaved area of the back of rabbits, produce a pronounced dermal inflammatory reaction. Extracts from nontoxic strains have no effect.

A total of 40 strains of *S. atra* was tested for toxicity. The fungi were grown in shaken cultures at 25° C. in a sterile medium containing 2 per cent milled wheat in water. This medium was used because it was observed in a preliminary test that one strain (1002) produced intradermally toxic substances within 7 days in this substrate. Each strain was observed grossly for rate of growth and degree of pigmentation over a period of 11 days. On the fourth, seventh, and eleventh days filtrates were removed for determination of *p*H and intradermal toxicity in rabbits. All strains grew well and formed a dark pigment by the end of the fourth day. The *p*H had changed from an initial value of 7.0 to one of approximately 4.5 by the fourth day, and was maintained at approximately this value during the remainder of the incubation period.

One strain (819) was investigated in great detail to obtain basic information for screening other strains for toxicity. This strain was cultured at various temperatures in static and shaken cultures containing organic and inorganic media, but did not produce substances toxic to

*S. *alternans* is no longer considered a valid species in the United States and England, and has been reclassified by Bisby[6] and White (personal communication) as *S. atra*.

mice, guinea pigs, rabbits, or chicks. Culture filtrates from 26 of 40 strains produced intradermal reactions in rabbits. Fifteen strains were selected for further study because their filtrates produced a pronounced inflammatory reaction. Of these, strains 821 and 1002 were finally selected for the animal toxicity experiments reported in this paper.

The strains were inoculated in replicates of 3 on sterile wheat straw in Fernbach flasks to determine whether the fungi would produce ether-extractable dermally toxic substances when cultured statically. On the eighty-fourth, one hundred and seventieth, and two hundred and sixty-third days of incubation the contents of one flask from each strain were removed, dried, ground, and extracted for 3 hours with anhydrous ethyl ether in a Soxhlet apparatus. The dry weight of ether-extractable substances in 10 ml. of the extract was determined. The color of the remaining ether extract was observed to determine whether a relationship existed between color and dermal toxicity; a known volume of olive oil was then added, and the residual volatile solvent was evaporated. A separate rabbit was used for testing each extract. For control purposes, a highly toxic suspension from strain 1069 was also tested on each rabbit. Each animal received 3 consecutive daily doses of 0.125 ml. of the oily suspensions.

As shown in TABLES 1 to 3 inclusive, neither color nor weight of ether-extractable material was indicative of dermal activity. Six of the fungi (strains 820, 873, 947, 1006, 1065, and 1069) produced pronounced dermal reactions on the eighty-fourth day of incubation. Two strains (820 and 1069) were highly toxic on the one hundred and seventieth day, and three strains (820, 1006, and 1069) produced necrotizing dermal lesions on the two hundred and sixty-third day of incubation.

Dermal Toxicity Studies (Strains 821 and 1002)

Dermal reactions. As previously stated, two toxic strains of *S. atra*, 821 and 1002, were selected for final toxicity studies. Mice, rabbits, horses, cattle, sheep, and swine were tested.

Dermal toxicity of ether extracts of straw on which strains 821 and 1002 had been cultured were first determined on the rabbit and the horse. The fungi were cultured on sterile, moist straw for approximately 20 days at 25° C.; then each sample of inoculated straw was removed, dried at room temperature, and ground to a powder in a Wiley mill. Thirty-five gm. of each was then extracted continuously for 4 hours with anhydrous ethyl ether in a Soxhlet apparatus. After suspending ether extracts in 10 ml. of olive oil the ether was removed by evaporation under reduced pressure. The oily suspensions were tested topically for toxicity. Three rabbits received a single application of 0.05 ml. of each suspension on a shaved area of the back. Two horses each received a 10 ml. application on a shaved and unshaved area of the neck.

TABLE 1

COLOR, WEIGHT, AND TOXICITY OF ETHER EXTRACTS FROM STRAINS OF *STACHYBOTRYS ATRA* INCUBATED 84 DAYS

Strain tested	*Color of extracts*	*Dose (mg./0.125 ml.)*	*Dermal reaction**	
			Test strain	*Control strain (1069)†*
816	yellow-green	1.9	SE	N
817	yellow-green	1.8	SE	N
820	green	1.3	N	N
873	amber	1.8	SE‡	SE‡
875	yellow	0.9	O	N
926	amber	1.4	SE	N
947	dark green	1.0	SE‡	SE‡
1006	green	1.4	N	N
1034	green	1.8	SE	N
1041	yellow	1.8	O	N
1065	green	2.4	SE‡	SE‡
1067	amber	1.4	SE	N
1069	amber	1.5	N	N

*Reaction observed 24 hours after the third daily dose: O denotes no reaction; SE, slight erythema; N, necrosis.

†The dosage of strain 1069 was 1.5 mg. in 0.125 ml. of olive oil.

‡Since extract from the control strain was known to produce a necrotizing lesion on a sensitive rabbit, the extracts from strains designated by the superscript are considered highly toxic.

TABLE 2

COLOR, WEIGHT, AND TOXICITY OF ETHER EXTRACTS FROM VARIOUS STRAINS OF *STACHYBOTRYS ATRA* INCUBATED 170 DAYS

Strain tested	*Color of extracts*	*Dose (mg./0.125 ml.)*	*Dermal reaction**	
			Test strain	*Control strain (1069)†*
816	yellow-green	0.80	O	N
817	yellow-green	0.92	O	N
820	green	0.78	N	N
873	amber	0.72	SE	N
875	yellow	0.64	SE	N
926	amber	1.21	SE	N
947	dark green	0.75	SE	N
1006	green	0.71	SE	N
1034	green	0.98	SE	N
1041	yellow	0.75	O	N
1065	green	1.06	SE	N
1067	amber	0.88	SE	N
1069	amber	0.84	N	N

*Reaction observed 24 hours after the third daily dose: O denotes no reaction; SE, slight erythema; N, necrosis.

†The dosage of strain 1069 was 0.84 mg. in 0.125 ml. of olive oil.

TABLE 3

COLOR, WEIGHT, AND TOXICITY OF ETHER EXTRACTS FROM STRAINS OF *STACHYBOTRYS ATRA* INCUBATED 263 DAYS

Strain tested	*Color of extracts*	*Dose (mg./0.125 ml.) olive oil*	*Dermal reaction**	
			Test strain	*Control strain (1069)* †
816	yellow-green	0.75	SE	N
817	yellow-green	0.91	SE	N
820	green	0.61	N	N
873	amber	0.67	SE	N
875	yellow	0.61	SE	N
926	amber	0.94	O	N
947	dark green	0.96	O	N
1006	green	0.64	N	N
1034	green	0.65	SE	N
1041	yellow	0.79	SE	N
1065	green	0.81	SE	N
1067	amber	0.90	SE	N
1069	amber	0.11	N	N

*Reaction observed 24 hours after the third daily dose: O denotes no reaction; SE, slight erythema; N, necrosis.

†The dosage of control strain 1069 was 0.84 mg. in 0.125 ml. of olive oil.

Application of strain 821 on the rabbits produced a hyperemia and edema at 24 hours, serum exudation at 48 hours, and necrosis at 72 hours (FIGURE 1). The suspension prepared from strain 1002 was nontoxic.

On horses, the suspension from strain 821 produced a slight inflammatory process consisting of slight hyperemia and slight sensitivity of the skin over the area of application (5 × 10 in.) at 24 hours. In 48 to 72 hours, hyperemia and sensitivity increased moderately. Edema and pinpoint serum encrustations formed and progressively increased until the area was covered with thick, soft, leathery crusts. Skin necrosis was observed during this process. After 72 to 96 hours, the skin wrinkled in a characteristic pattern, made up of parallel folds along a line midway between the short and long axis of the neck (FIGURE 2). These developed slowly from barely visible folds to pronounced wrinkles over the entire area of application. In most cases cross stria perpendicular to the parallel skin folds developed and produced a crosshatched pattern. The animals became depressed and assumed a staring, apathetic attitude, sometimes resting their heads on and against the stall. Complete anorexia was never observed during this period of malaise, which normally disappeared 24 to 48 hours later. Slightly increased lacrimation was usually observed. The conjunctival mucous membrane was slightly to profusely injected and slightly to moderately icteric. The nasal mucous membranes were slightly to moderately injected. The oral mucous membrane remained normal. All signs of inflammation and wrinkling of the skin disappeared

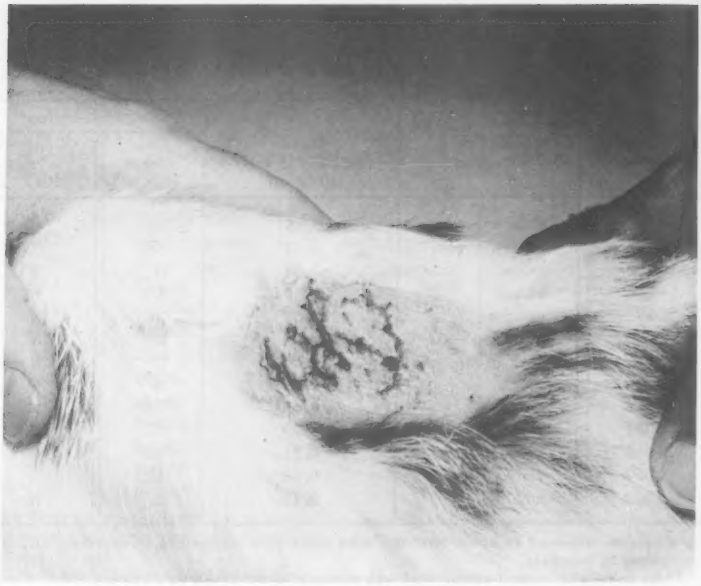

FIGURE 1. Topical necrosis as seen on skin of a rabbit 72 hours after receiving 0.125 ml. of an ether extract of strain 821 of *S. atra* in olive oil.

within 5 to 7 days. During the development and healing of the skin lesion, there was no evidence of hair loss or signs of itching. The suspension prepared from strain 1002 was dermally nontoxic for horses.

Strain 1002 became highly toxic after repeated culturing on straw. Extracts of straw on which this strain had been cultured for 40 days at 25° C. were also tested for dermal toxicity on the rabbit, calf, and horse. The animals received applications of the extracts for 3 consecutive days. The rabbit received 0.125 ml. doses over a shaved area of 40 sq. mm. on the back; the calf and horse received 10 ml. amounts on an unshaved area of 5 × 10 in. on the side of the neck. An acute inflammatory reaction was observed in all 3 animal species within 24 hours after the first dose. This was characterized by profuse hyperemia, gross edema, parallel skin folding, increased sensitivity, systemic depression, and anorexia (FIGURES 2, 3, and 4). During the 72- to 96-hour stage, the skin began sloughing in the area of necrosis. The appetite gradually returned to normal. Lacrimation was profuse in the calf and moderately increased in the horse. None was observed in the rabbit.

In the horse and calf, the conjunctival mucous membranes were grossly injected and slightly icteric during the 24- to 96-hour period. In the calf, the pulse rate increased to 96 per min., but the temperature remained

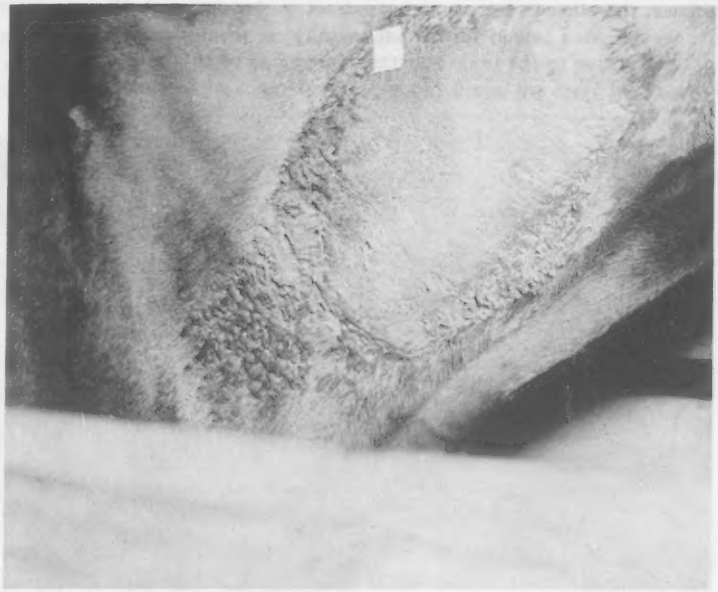

FIGURE 2. Topical necrosis as seen on skin of neck of a horse 72 hours after application of 10 ml. of ether extract of strain 1002 in olive oil.

normal. Respirations of the Cheyne-Stokes type increased to 120 per min. The cellular blood picture remained within normal limits in all animals.

Oral administration reactions. The dermally toxic ether extract from strain 1002 was tested orally for toxicity in the horse and calf. A preparation containing 2.0 gm. of ether-extracted material in 250 ml. of olive oil was administered to a horse by stomach tube in a single dose. A calf received by the same route, over a period of 20 days, 22.5 gm. of the toxic-extracted substance in 2250 ml. of olive oil. During the first 5 days following administration of the oily suspension, both animals lacrimated moderately, became slightly depressed, and subsequently returned to normal.

Effect of incubation time on production of dermally-toxic substances. To determine the optimal incubation period for production of the dermally toxic ether-extractable substance by strain 1002 under static conditions, the fungus was inoculated on sterile wheat straw in 11 Fernbach flasks and incubated at 25° C. At various intervals, the contents of 1 flask were removed, dried, and ground to a powder, and 35 gm. was extracted continuously with 300 ml. of anhydrous ethyl ether in a Soxhlet apparatus. After determination of the dry weight of ether-soluble material of a 10 ml.

volume, the ether in the remaining extract was evaporated. The resultant suspension was tested for dermal toxicity on rabbits previously shown to be sensitive to the toxic substance produced by this strain of *S. atra*. Each rabbit received one 0.125 ml. dose on each of 3 consecutive days.

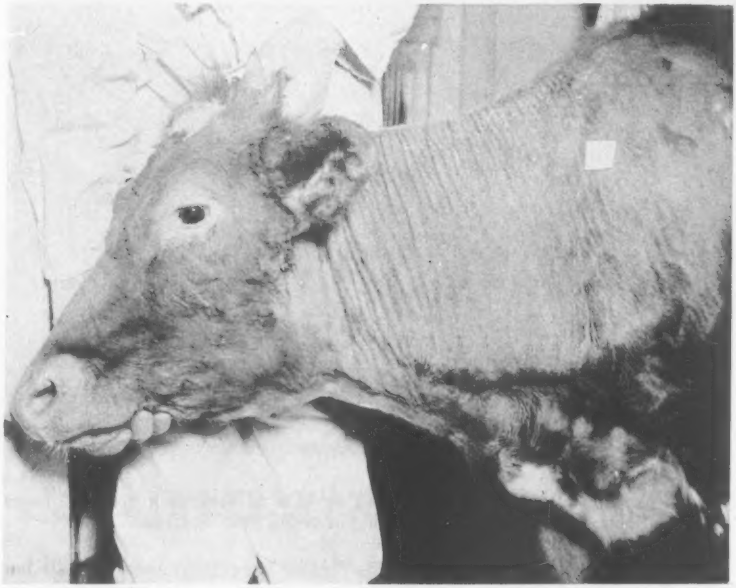

FIGURE 3. Parallel skinfolding on neck of calf following one 10 ml. application of ether extract in olive oil prepared from straw incubated with *S. atra* 1002.

Data presented in TABLE 4 indicate that the dermally toxic substance began to form within 10 days. At 19 days it attained a good level (as evidenced by a necrotic lesion on the rabbit), which was maintained for a minimum of 414 days. Although the weight of the ether-extractable substances, in general, decreased from the one hundred and ninth day of incubation, the severity of the dermal inflammatory reaction was not reduced.

Effect of time of extraction on recovery of ether-soluble and dermally toxic substances. To determine the optimal time required to extract ether-soluble substances from straw on which the fungus had been cultured, 3 samples of ground straw of known weight were extracted continuously with 300 ml. volumes of anhydrous ethyl ether for 9, 15, and 60 min., respectively. A 10 ml. sample of each ether extract was removed for determining the weight of ether-extractable substances present. The remaining ether extracts were suspended in olive oil and

TABLE 4

EFFECT OF INCUBATION PERIOD OF STRAW INOCULATED WITH *STACHYBOTRYS ATRA* 1002 AND DERMAL TOXICITY OF EXTRACTS

Incubation period (days)	*Wt. (mg.) Ether-extractable material/0.125 ml.*	*Dermal reaction**
10	0.6	SE
19	1.3	N
25	1.6	N
33	1.6	N
47	1.7	N
74	1.6	N
109	1.0	N
125	0.9	N
160	0.8	N
220	0.8	N
414	0.4	N

*Reaction observed 24 hours after 3 daily doses: SE denotes slight erythema; N, necrosis.

tested topically for dermal reaction on a sensitive rabbit. Each rabbit received 1 dose of each extract in 0.125 ml. of suspension. Results presented in TABLE 5 indicate that most of the ether-soluble substances were removed within 15 min., since prolonging the time of extraction to 60 min. did not increase the amount of ether-extractable substances. The 3 extracts were equally toxic, as evidenced by a moderate erythema at the site of application.

TABLE 5

EFFECT OF TIME OF EXTRACTION OF STRAW INFECTED WITH *STACHYBOTRYS ATRA* ON RECOVERY OF ETHER-SOLUBLE SUBSTANCES

Extraction time (min.)	*Wt. of straw smpl. (gm.)*	*Ether solubles*		*Reaction**
		Per cent extracted	*Wt. (mg.)/ 0.125 ml.*	
9	37.30	0.07	0.600	ME
15	32.33	1.08	0.825	ME
60	30.05	1.02	0.700	ME

*Dermal reaction on rabbits: ME denotes moderate erythema.

Another sample of toxic straw, weighing 27.4 gm., was extracted with anhydrous ether to determine the time required for removal of dermally toxic substances. At hourly intervals, for 7 hours, the ether containing the extract was removed and the weight of extractable substances determined in a 10-ml. sample. The remaining ether solution was suspended in olive oil, the solvent evaporated, and the oily suspensions tested topically for dermal reaction on a sensitive rabbit.

As shown in TABLE 6, the dermally toxic substance was removed after 3 hours of continuous extraction. The amounts recovered thereafter were insufficient to produce a dermal reaction. Approximately 80 per cent of the ether-extractable material was removed during the first hour of extraction.

TABLE 6

INFLUENCE OF TIME OF EXTRACTION OF *STACHYBOTRYS ATRA* STRAW ON REMOVAL OF DERMALLY TOXIC SUBSTANCES

Extraction time (hr.)	*Ether solubles*		*Reaction**
	Per cent extracted	*Wt. (mg.)/ 0.125 ml.*	
1	1.28	1.750	PE
2	0.12	0.577	ME
3	0.08	0.117	ME
4	0.05	0.061	O
5	0.05	0.061	O
6	0.03	0.037	O
7	0.03	0.036	O

*Dermal reaction on rabbits: PE denotes pronounced erythema; ME moderate erythema.

Quantitative determination of toxicity of crude ether extracts. Strains of *S. atra* varied in their ability to produce toxic substances, as shown in TABLES 1 to 3. Extracts of straw on which some strains had been cultured when applied topically to rabbits were nontoxic. Some extracts produced slight erythema, others produced a moderate erythema, and still others necrotic lesions. The quantitative dermal toxicity of crude ether extracts of strain 1002 was determined. An extract of straw on which the fungus had been cultured for approximately 60 days at 25° C. was dried and extracted for 3 hours with anhydrous ethyl ether. After evaporation of the solvent, a known weight of the residue was dissolved in hot ethanol and then suspended in olive oil. The suspension was diluted serially 7 times in tenfold dilutions in olive oil to give a final concentration of 0.000175 μg. per 0.125 ml. dose. The suspensions were then topically applied daily for 3 successive days on a shaved area of the back of a sensitive rabbit. Suspensions containing 17.5 μg. or more per dose caused necrosis; those containing 0.00175 μg. or more produced a slight to pronounced hyperemia; and the greatest dilution showed no effect 24 hours after the third dose.

Concentration of dermally toxic substances. A 1-lb. sample of dermally toxic straw was extracted continuously with anhydrous ethyl ether for 3 hours. The ether was evaporated and the residue, which was dermally toxic and consisted of a mixture of crystals and oil, was dis-

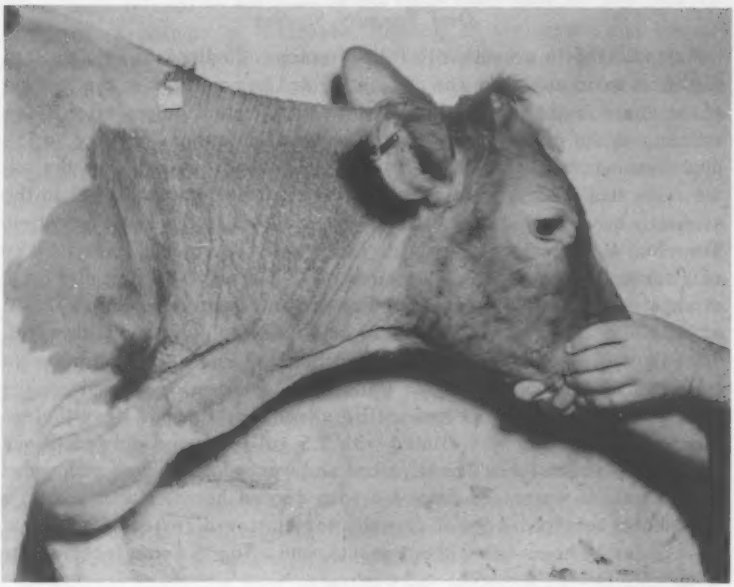

FIGURE 4. Serum incrustations on neck of calf following second topical application of 10 ml. of ether extract in olive oil prepared from straw incubated with *S. atra* 1002.

solved in warm carbon tetrachloride. The solution was cooled to 4° C. and a precipitate that formed was filtered. The carbon tetrachloride filtrate, which contained much oil, produced a moderate erythema on the rabbit. The precipitate, which was repeatedly dissolved in hot ethanol and precipitated in cold ethanol, was dermally nontoxic when dissolved in warm ethanol. The fraction soluble in cold ethanol was toxic. The toxic alcoholic fraction was evaporated to dryness, and the gummy residue obtained was dissolved in hot carbon tetrachloride. This solution was passed over a column of activated alumina, and the column was washed with 100 ml. volumes each of carbon tetrachloride, *n*-heptane, benzene, chloroform, and hot ethanol. Each eluate was evaporated to dryness, and the respective residue was dissolved in 20 ml. of warm ethanol and tested for dermal toxicity on a rabbit.

Activated alumina absorbed the dermally active substance. Trace quantities of it, evidenced by a very slight hyperemia on rabbits, were removed by carbon tetrachloride, *n*-heptane, and benzene. Chloroform removed much of the active fraction, which was yellow in color. This fraction produced a necrosis on the rabbit. Hot ethanol removed a light brown pigment, which was as active as the chloroform fraction.

Oral Toxicity Studies

As indicated in a section to follow, autopsy findings of animals that died from toxic straw showed congestion and hemorrhages in the mucosa of the stomach and in that portion of the small intestine posterior to the opening of the common bile duct. The small intestine, anterior to the duct opening, was essentially normal. This observation led to the assumption that a toxic substance was digested and was absorbed in the stomach, concentrated in the liver, and excreted in the small intestine. Therefore the effects of pepsin digestion were determined on solubility of a toxic substance present in straw. A 2-lb. sample of dermally-toxic straw was ground to a powder, and digested with porcine pepsin at 37° C. at *p*H 1.5 in 2 gal. of water, filtered, and repeatedly washed with water. The digestate and washings were concentrated under reduced pressure to approximately one-half gal. During the evaporation procedure, the liquid was accidentally heated until a slight char formed. Nevertheless the remaining liquid was diluted with 1.5 gal. of water and fed to one horse by stomach tube. The digested and washed straw was suspended in 1.5 gal. of water and force-fed to a second horse. For controls, a third horse received 2 lb. of dermally toxic straw digested at *p*H 1.5 at 37° C. for 24 hours but without pepsin, and a fourth horse received the nonpepsin-treated digestate and washings.

The first animal showed acute toxic symptoms, characterized by depression, anorexia, profuse perspiration, congestion, and icterus of the conjunctival mucous membranes. This condition existed for 4 days. The third horse also demonstrated acute toxic symptoms and was found dead 17 hours later. The second and fourth horses remained normal. Results of this experiment indicate that pepsin digestion renders the toxic material contained in infected straw water-soluble and therefore available for absorption through the gastric mucosa.

Toxicity of strain 1002-infected straw for horses. Straw on which strain 1002 had been cultured for approximately 60 days at 30° C. was dried at room temperature, ground to a powder in a Wiley mill, and fed to horses. Ether extract prepared from a sample of the ground straw was dermally toxic on the rabbit.

Free-feeding. Two horses previously starved for 48 hours were subjected to free-feeding of approximately 0.25 lb. of the ground straw mixed with 0.25 lb. of cane sugar and 1 gal. of oats. The horses readily ate only 3 or 4 mouthfuls of the material. Apparently, the odor and taste were not objectionable, but after the fourth mouthful, some unpleasant sensation occurred, because the animals refused to eat more of the toxic mixture. Within 24 hours, the animals began to salivate and lacrimate, and the buccal mucosa became moderately inflamed and congested. In 48 to 72 hours, the mucous membranes became swollen, necrotic, and started to slough. The commissures of the lips cracked, and the conjunctival and nasal mucous membranes became grossly injected. Since

the animals refused to eat more of the toxic feed, they did not develop additional symptoms of toxicosis. Healing of the mouth and buccal cavity occurred in 7 to 9 days after initial exposure to the toxic straw.

A third horse, after considerable inducement, consumed over a 10-day period, approximately 0.5 lb. of toxic straw in blackstrap molasses and oats. During this period, the animal developed signs and lesions similar to those observed in the 2 horses described in the preceding paragraph. On the eleventh day, the horse was unable to rise and showed an increased respiration rate (58 per min.) and rise in body temperature to 105.8° F. Thereafter, the horse developed convulsions and was destroyed for humane reasons. Post-mortem and histopathological findings were the same as described in the section to follow for horses that died from forced-feeding of toxic straw.

Forced-feeding. Two horses were fed by stomach tube 1.5 lb. and 2.0 lb., respectively, of toxic straw in an aqueous slurry. Six hours after the administration of the slurry, the animals showed evidence of colic and died 17 hours after feeding.

Gross findings at autopsy were as follows. The pleura was injected, edematous, and contained diffuse pinpoint hemorrhages varying in diameter from 1/8 in. to 5 in. The diaphragm was also injected and hemorrhagic. Areas of congestion, atelectasis, hemorrhages, and emphysema were present along the ventral edges of the lung. Blood-tinged, frothy fluid partially filled the lumen of the trachea, bronchii, and bronchioles. Diffuse hemorrhages and congestion were visible in the pericardium, epicardium, myocardium, and endocardium. The liver was so greatly engorged that large quantities of blood were released upon sectioning. The tracheal, mediastinal, and intestinal lymph nodes were hemorrhagic, edematous, and greatly enlarged. Profuse hemorrhages and edema were noted in the adrenals. The mucosa of the fundic portion of the stomach revealed severe injection, hemorrhages, and the presence of a brownish-purple pigment. The mucosa of the small intestine posterior to the common bile duct opening was grossly injected, swollen, and readily peeled from the submucosa although, anterior to the duct opening, the mucosa was only moderately injected. In addition, the caecum was injected and showed evidence of purpura. The omentum contained spotty areas of hemorrhage and congestion. Subcapsular hemorrhages varying in diameter from 1/2 in. to 2 in. were readily visible in the cortex of the kidney.

Histopathological findings included the following. The lungs showed congestion, hemorrhages, and atelelectatic areas in addition to pneumonia. Hemorrhages were also noted in the heart and its covering. The sinusoids of the liver were greatly congested, and there was great engorgement and thrombi formation within the central veins. Some of the liver cells revealed vacuoles (FIGURE 5) that contained pleomorphic, nonspecific bodies that stained red with the Giemsa stain. Massive hemorrhages and congestion were noted throughout the cortex of the kidneys, and the

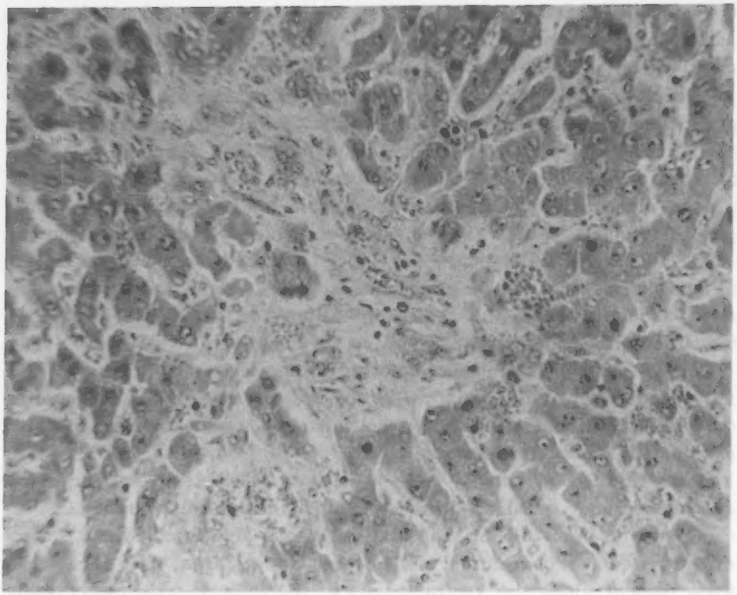

FIGURE 5. Liver showing vacuolation of cytoplasm and areas of necrosis (×400).

epithelium of the tubules was separated from the basement membrane, appearing as epithelial casts in the tubules (FIGURES 6 and 7). Congestion and hemorrhages were observed in the fundus of the stomach and in the mucosa of the small intestines. Areas of necrosis in the zona fasiculata and hemorrhages in the cortex and medulla of the adrenals were noted. Extensive hemorrhages in the mediastial and tracheal lymph nodes were present with necrosis of the lymphoid follicles. The thyroid was congested and hemorrhagic.

Oral toxicity of strain 1002-infected substrates for the calf, sheep, hog, and mouse. Toxic straw similar to that used in the previous section was suspended in water and fed by stomach tube to a calf, sheep, and hog. Oats on which the fungus had been cultured for 21 days were fed *ad libitum* to mice. A 250-lb. calf received 6 lb. of straw over a period of 14 days; a 130-lb. ewe received 1/3 lb. in a single dose; a pig, weighing 125 lb., received 1/5 lb. in a single dose; and 10 mice were fed 20 gm. each for 7 days.

Four days after initiation of feeding, the calf developed slight depression and lacrimation. After 6 to 8 days, anorexia, watery stools, and greater depression were observed. The animal progressively developed greater malaise, dehydration, and diarrhea. Agranulocytosis became evi-

dent on the ninth day and continued to increase as indicated in TABLE 7. A pronounced decrease in retraction of the blood was observed, and coagulation time changed from 9 min. on the first day before the start of the experiment to 19 min. on the third day preceding death. Forty-eight hours before death, the temperature rose to 107.2° F. In the terminal stages, the calf evidenced respiratory embarrassment and dyspnea and died on the seventeenth day of the experiment.

TABLE 7

WHITE BLOOD CELL COUNT OF CALF SUBJECTED TO REPEATED FEEDING OF STRAW INOCULATED WITH *STACHYBOTRYS ATRA* 1002

		Blood Count									
Days of test	Infected straw fed (lb.)†	Leukocytes per ml.	Neu.	Fil.	Band.	Juv.	Differential* Lymphocytes			Mono. and Gran.
							Large	Small	Total	
0	0	4,500	50	47	2	1	14	32	46	4
1	0.5	3,750	54	48	4	2	16	29	45	1
2	0.5	2,800	49	46	2	1	24	21	45	6
3	0.5	2,300	53	47	3	3	9	30	39	8
4	0.5	1,350	55	28	15	12	25	12	37	8
5	0.5	650	65	37	8	20	10	20	30	5
6	0.5	1,100	52	43	7	2	2	36	38	10
7	0.5	1,050	57	49	2	6	5	37	42	1
8	0	550	61	41	15	6	7	30	37	2
9	0.5	650	63	38	15	10	14	20	34	3
10	0.5	700	65	40	13	12	20	22	42	1
11	0	750	67	42	11	14	16	14	30	3
12	0	850	68	44	18	6	18	12	30	2
13	1.0	650	74	51	14	9	15	10	25	1
14	0.5	400	72	43	21	7	19	17	36	2
15	0	150	2	2	0	0	76	22	98	0
16‡										
17‡										

*Abbreviations were used as follows: Neu. denotes neutrophiles; Fil., filaments; Band., bands; Juv., juveniles; Mono., monocytes; Gran., granulocytes.

†This calf had been previously fed inoculated straw (strain 1002) and showed agranulocytosis. At the beginning of this experiment the white cell count was in the upward trend of normalcy.

‡No blood counts made on days indicated. Calf died on seventeenth day.

Gross autopsy findings, for the most part, were the same as those observed in the horses. In addition, tissues in the submaxillary space were edematous and contained a reddish, straw-colored fluid over an area 8 in. wide by 14 in. long. The gall bladder was distended and showed bile pigment inbibition of the mucosa and serosa. In the adrenals, the cortical granular lipoid material was almost completely depleted. The spleen was atrophied and contained many subcapsular pinpoint hemorrhages. The mucosa of the small intestine was edematous and injected and contained blood-tinted material in the lumen.

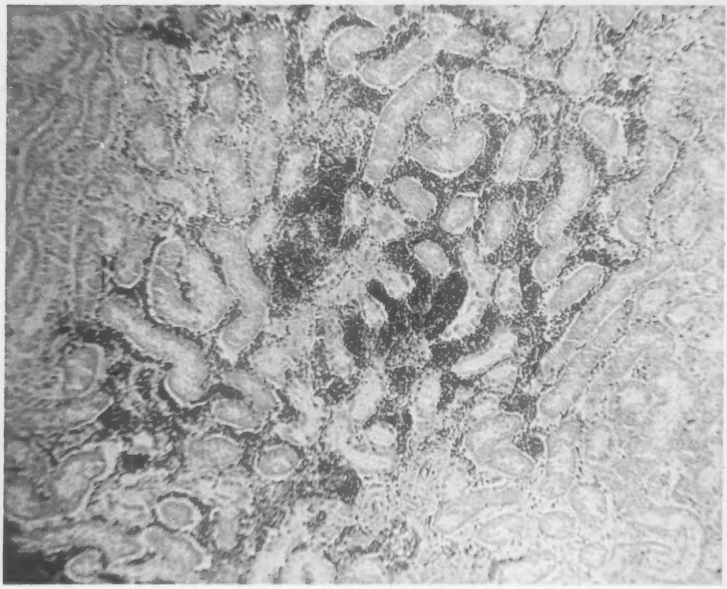

FIGURE 6. Areas of hemorrhages and congestion as seen in the kidney (×400).

On histopathological examination, the kidneys showed dilatation in the collecting tubules. Sinusoids of the liver were congested and, in the gall bladder, there was submucosal edema and presence of red-staining, refractile bodies of varying sizes. The spleen was aplastic, dystrophied, and showed diminution of the lymphoid elements and presence of many blastic cell forms.

The ewe, first observed 12 hours after feeding, was prostrate and breathing heavily. The animal progressively became weaker and died 13 hours later.

The pig was found dead 17 hours after feeding.

Mice showed atony and slight depression and cachexia on the third day of feeding. Cachexia progressively became more pronounced and all animals were dead on the seventh day.

Gross and histopathological findings in the ewe, hog, and mice were essentially the same as those observed in the calf.

DISCUSSION

Fungi belonging to the genus *Stachybotrys* are widely distributed in nature. Although these fungi can frequently be isolated from straw, hay, and other crop residues, their significance in mycotoxicoses in animals

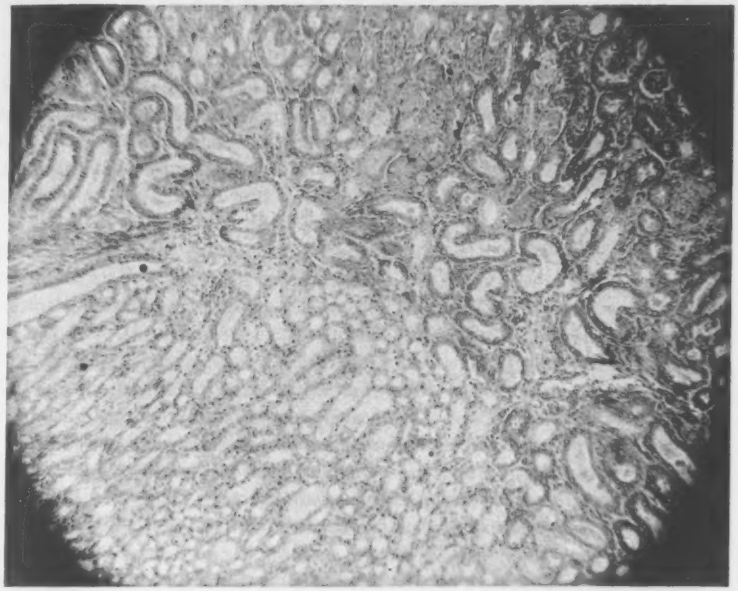

FIGURE 7. Kidney showing tubular dilation (×200).

has not been studied in the United States. In fact, the economic significance of saprophytic fungi, in general, until recently has received very little study from the standpoint of animal health. Forgacs *et al.*[7] isolated a strain of *Aspergillus clavatus* from a pelleted feed that produced hyperkeratosis in calves that consumed the feed. Clinical, gross, and histopathological findings in calves that consumed corn on which the fungus isolated from the feed had been cultured, showed typical signs of bovine hyperkeratosis. Similarly, Carll and his co-workers[8] found that a strain of *Aspergillus chevalieri* isolated from a hyperkeratosis-producing lot of bread crumbs, when cultured on a similar substrate and fed to calves produced manifestations of the toxicosis. According to Carll and his associates,[9] substrates on which a nonpathogenic strain of *Aspergillus fumigatus* had been cultured were toxic to a calf. Sippel *et al.*[10] and Burnside *et al.*[11] found that a strain each of *Aspergillus flavus* and *Penicillium rubrum* produced toxic manifestations in small and large animals. The fungi had been isolated from mouldy field corn that caused severe losses in pigs that foraged the grain. More recently Forgacs *et al.*[12-14] presented evidence that various saprophytic fungi belonging to the genera *Alternaria, Aspergillus, Paecilomyces,* and *Penicillium* that had been isolated from broiler houses where poultry hemorrhagic

syndrome was endemic, were toxic. When cultured on whole-grain corn, scratch grain, or normal broiler mash containing no coccidiostat or antibiotic, these fungi, under proper environment, produced substances that under both laboratory and field conditions were toxic to chicks and older birds. Clinical gross and histopathological manifestations resembled those observed in field cases of the hemorrhagic syndrome.

Early observations by Schofield[15,16] indicated that spoiled sweet clover hay *(Melilotus alba* and *M. officinalis)* that caused a hemorrhagic disease in cattle was highly contaminated with a dark fungus growing within and on the stem. In addition, Schofield isolated an *Aspergillus* species that, when cultured on sweet clover hay and fed to rabbits, produced symptoms resembling the bovine sweet clover disease. He made no mention of isolating the dark dematiaceous fungus. The techniques used by Schofield for isolating fungi probably would have failed to recover *Stachybotrys sp.* from his specimens even had they been present. His techniques would encourage more luxuriantly growing fungi such as *Penicillia, Aspergilli,* or members of the *Mucorales,* to multiply and readily overgrow the surface of the agar plates, thereby masking the growth of slower-growing fungi, such as *Stachybotrys.* It appears that the role of such fungi as *Stachybotrys* and members of the related genus *Memnoniella* should have received more attention in sweet clover poisoning.

Marked differences were noted in the ability of stock strains of *S. atra* to produce the toxic factors. This was evidenced by the fact that cultural filtrates of only 26 of 40 studied produced intradermal reactions in rabbits on initial screening and that only 15 of the 26 produced marked inflammatory reactions. Strain 1002, an example of a strain previously shown to be dermally nontoxic, became highly toxic after repeated transfer on straw. Repeated subculturing on straw gave rise either to a mutant that acquired the ability to produce the dermally toxic factor, or activated a dormant metabolic system responsible for production of the toxic principle. The successful isolation of a toxic factor from strain 1002 illustrates the need for carefully screening strains of *S. atra* isolated from suspected cases of fungotoxicosis before eliminating them as the causative agent. Ether extracts of straw on which strain 1002 had been cultured, when applied topically on the horse, calf, and rabbit, produced essentially the same syndrome, indicating that dermal toxicity may not be restricted to any one species of animal. However, as indicated in TABLE 1, there exists a variation in susceptibility of rabbits to the dermally toxic substance.

Although ether extracts of toxic straw, when suspended in olive oil and when applied topically on the rabbit, horse, and calf, produced severe local reactions (FIGURES 1, 2, and 3), and a slight systemic depression, no pronounced systemic toxicosis was observed when a similar suspension was administered orally to the horse and calf. Apparently, either the oil interfered with absorption of the toxic substance

during passage through the gastrointestinal tract, or the dermally toxic factor may be different from the orally toxic substance. According to Vyshelesski,[4] straw on which toxic strains has been cultured and that has been continuously extracted with anhydrous ethyl ether still contains the orally toxic principle; this would suggest that the dermal and oral toxic factors might be separate entities. Nevertheless, he found a correlation between dermal and oral toxicity. Perhaps both are produced simultaneously.

Two horses that were fed *ad libitum* straw on which strain 1002 had been cultured developed the first stage of the typical form of stachybotryotoxicosis. The first two horses ate only 3 or 4 mouthfuls and thereafter refused to eat more of the toxic straw, possibly due to a burning sensation similar to that described by Mayer[17] and Gajdusek[5] in humans who complained of a sensation similar to a mild scalding after eating the first mouthful of cereal or by-products of cereals infected with a toxic fungus, *Fusarium sporotrichoides*.

The atypical or shocking form of stachybotryotoxicosis was produced in 3 horses. One animal that had been induced to eat 0.5 lb. of toxic straw and 2 horses that had been force-fed by stomach tube 1.5 and 2.0 lb. respectively of the ground straw in an aqueous slurry developed acute manifestations of systemic toxicity. Gross and histopathological findings were the same as those described by Vertinskii,[1] Vyshelesski,[4] Drobotko,[2,3] and others.

Although not described in this paper, efforts were made to produce the second stage of the typical form of stachybotryotoxicosis. Three horses that had been force-fed sublethal quantities of toxic straw developed the second stage of the toxicosis. In addition, a calf that had been fed 6 lb. of toxic straw over a period of 14 days showed an increase in blood clotting time and, as indicated in TABLE 7, developed an agranulocytosis, both manifestations being characteristic of the second stage of the toxicosis.

In the past, the horse was considered the only large animal susceptible to the toxic substance produced by selected strains of *S. atra*.[1-4] Our studies have shown that the calf, sheep, pig, and mouse are also susceptible to the toxic principle. The calf, however, appears to be more resistant than the other animals. Whereas only 0.5 to 2.0 lb. of toxic straw caused death in horses within 17 hours, 6 lb. were required to produce death in the calf on the seventeenth day.

Perhaps discrepancy between claims made by the European workers and our findings may be attributed to differences in physiological characteristics among the strains of fungi used. Based on the dermal inflammatory reaction of ether extracts prepared from straw on which 13 strains of *S. atra* had been cultured, it has been demonstrated that there exists a variation among strains of the fungus in ability to produce the dermally toxic substance. Of 13 strains tested, only 3 were highly toxic after 263

days of static incubation on wheat straw (TABLE 3). Eight strains produced a slight hyperemia, and 2 were nontoxic.

Gross and histopathological examination of animals that died after being fed dermally toxic straw showed massive hemorrhages and congestion in the stomach of the pig and horse and in the abomasum of the calf and sheep. Anterior to the common bile duct opening, the small intestine appeared essentially normal; whereas, posterior to the duct opening, the intestine was hemorrhagic and congested, indicating that digestion and absorption of the toxic substance occurred in the stomach. This supposition was substantiated by digesting dermally toxic straw with pepsin at *p*H 1.5 for 24 hours at 37° C. This procedure rendered the toxic substance water-soluble. When force-fed to a horse, the digestate obtained from straw treated in this manner produced some of the clinical manifestations of the shocking form of stachybotryotoxicosis. The digested and washed straw was nontoxic. Digestion of toxic straw at *p*H 1.5 without pepsin did not remove the toxic substances. Due to circumstances beyond our control, the effect of pepsin digestion of dermally toxic ether extracts was not performed. This procedure might have differentiated between the dermally and orally toxic factors.

Information published by Caskey,[18] Cover *et al.*,[19] and Yacowitz *et al.*[20] indicates that there exist two types of a poultry hemorrhagic syndrome. One responds to vitamin K therapy; the other does not. Forgacs *et al.*[12-14] have demonstrated that certain saprophytic fungi, when inoculated on suitable substrates and fed to day-old chicks and older birds, will produce clinical, gross, and histopathological findings resembling those observed in broilers afflicted with the hemorrhagic syndrome. They did not publish hematological findings, but their unreported data indicate that blood-clotting time did not appear to be prolonged. This information would indicate that certain toxic fungal substrates do not interfere with the prothrombin. On the other hand, data presented in this publication, as well as evidence presented by Vertinskii,[1] Vyshelesski,[4] Mayer,[17] and Gajdusek,[5] indicate that the *Stachybotrys* and the *Fusarium* fungi produce substances antagonistic to vitamin K metabolism. Therefore, the possibility that fungi may be a factor in the production of both types of poultry hemorrhagic syndrome is plausible.

European workers have given considerable attention to mycotoxicoses in animals and humans (Serkisov[21]), but the problem has received very little consideration in the United States, and it appears that the role of toxic fungi in toxicoses of unknown etiology in this country deserves more study.

SUMMARY

A total of 40 strains of *Stachybotrys atra* were investigated for ability to produce substances that are toxic for animals. Cultural filtrates from 26 of these produced intradermal reactions in rabbits, and 15 produced

marked inflammatory reactions. Two strains, 821 and 1002, which were studied in detail, produced ether-extractable substances dermally toxic for rabbits, cattle, and horses. The reactions were characterized by hyperemia, edema, and subsequent necrosis.

The dermally toxic substance was produced by strain 1002 on wheat straw within 10 days and was still present at the end of 414 days of incubation. Most ether-soluble substances were removed from the toxic straw within 15 min., but 3 hours' extraction was necessary to remove the dermally toxic factor. This toxic factor was absorbed by activated alumina, was eluted with chloroform or hot ethanol, and was active at a concentration of 0.00175 μg./0.125 ml.

When administered orally in olive oil, the dermally toxic substance produced essentially no toxic manifestations in the calf and horse, indicating a possible interference of the oil with gastrointestinal absorption of the toxic substance.

The dermal toxicity of ether extracts of straw on which *S. atra* were cultured varied with the strain of fungus tested. An orally toxic factor present in infected straw was water-insoluble, but digestion with pepsin at *p*H 1.5 rendered it water-soluble.

Straw on which strain 1002 had been cultured was toxic when fed to the horse, calf, hog, and sheep. Oats on which this strain had been cultured were toxic when fed to mice. When fed to horses, the 1002-infected straw produced the atypical form of stachybotryotoxicosis, characterized primarily by a hemorrhagic diathesis and plethora. Sublethal amounts, when force-fed to a calf over a period of 14 days, produced a leucocytosis, subsequent leucopenia, and prolonged blood-clotting time.

Some strains of *S. atra* lack the ability to produce toxic substances on initial screening, but by serial passage on suitable media develop the property. This fact emphasizes the need carefully to screen strains isolated from suspected cases of fungotoxicosis for toxin-producing variants before eliminating them as causative agents.

ACKNOWLEDGMENTS

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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF BIOLOGY

IONIZING RADIATION AS A TOOL IN BIOLOGICAL RESEARCH*

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I. *Introduction*

This paper deals with two rather diverse areas of science: (1) the purely physical phenomena associated with the interaction of ionizing radiation with matter, and (2) the exceedingly complex processes associated with a living organism.

The science of "radiobiology" was born when living tissue was chosen as the target material for ionizing radiation. Shortly after the discovery of X rays by Roentgen (1895) and radium by the Curies (1898), it was learned that these forms of radiant energy exerted effects peculiarly destructive to living matter. This motivated further experiments and opened up a whole new field of exploration in biology. During the earlier years of this new science, investigations involving the use of ionizing radiation were concerned largely with the destruction of malignant tissue. In the present atomic era the uses of radiation energy have become legion, transcending its purely scientific applications as a tool for radiobiological research. During and since World War II, new areas of radiation research have been opened up in many fields, directed toward such diametric goals as the immediate destruction of man by atomic explosions and the prevention or cure of radiation injuries. Ionizing radiations are also widely used in industry and for military purposes.

In recent years considerable attention has been focused upon studies of the effect of radiation on fundamental life processes, such as cell division and genetic changes, because of their importance in elucidating the mechanisms involved in mutation, carcinogenesis, aging, and death induced by ionizing radiation. Furthermore, ionizing radiation is used not only as a means of triggering various biological reactions, but also as a powerful analytic tool in the form of radioactive tracers. This particular use of radiation sources has extended the scope of observability of specific cellular processes by another order of magnitude, particularly where the dynamic aspects of cellular functions are concerned. Consequently, the field of radiobiology embraces important segments of many scientific disciplines. In addition to biology, the fundamental concepts and techniques of physics, chemistry, biochemistry, physiology, pathology, and genetics are essentially involved.

*This paper, illustrated with slides, was presented at a meeting of the Section on January 13, 1958.

The selection of test material in radiobiological research generally depends on the specialty of the investigator. For example, *Drosophila* eggs and *Paramecia* are used mainly by geneticists because of their rapid rate of multiplication. Plants are preferred for studies of the nuclear apparatus since they possess large chromosomes; for physicobiological measurements, unicellular organisms such as bacteria, viruses, and yeast are employed.

Since a true appreciation of the value and versatility of ionizing radiation as a tool in biological research necessarily requires a basic knowledge of the mechanisms involved, it will be instructive to review briefly the important physical properties of ionizing radiation and the mechanisms of its interaction with biological systems.

II. *Ionizing Radiations and Their Action on Biological Systems*

Ionizing radiation and its sources. The significant property of the type of radiation used in biological research is its extreme penetrating power. This is in contrast to the other well-known forms of radiant energy (infrared, optical, and ultraviolet rays), since ionizing radiations are capable of penetrating deeply within the organism, whereas the less penetrating rays are entirely absorbed on or near the surface.

The penetrating radiations that have been used to study biological systems fall into two categories: (1) electromagnetic radiation of very short wave length, for example, X rays and gamma rays from radium and from other radioactive sources; and (2) corpuscular radiation, which consists of high-velocity particles, such as alpha and beta particles, protons, and neutrons. Of these two categories, the first is by far the most common source used for external irradiation of biological systems. This is due principally to the ease with which a quite homogeneous beam of known energy can be generated. Gamma rays with energies in excess of 1 mev are also utilized. The latter were originally obtained from radium and other naturally radioactive elements, but with the development of new machines such as the van der Graff generator, the linear accelerator, the betatron, and the synchotron, artificial generation of radiation in the mev energy range became a practical possibility. Furthermore, the radiation generated by these machines has the advantage of greater homogeneity than that obtained from the natural radioactive elements. Artificially produced radioactive elements have also proved useful as sources of radiation; for example, Cobalt 60, produced in a nuclear reactor, provides a useful source of gamma rays of energies between 1.1 and 1.3 mev. Its use in radiobiological research and therapy is increasing steadily.

In the tracer field the source of radiation is, of course, a radioactive isotope. These isotopes are chemically identical with their stable counterparts, having the same atomic number but differing in atomic mass. Thus

they may be substituted in place of many of the analogous stable chemical elements that make up living matter without disturbing the physicochemical processes in which they are involved. These isotopes decay at characteristic rates and emit gamma rays, corpuscular rays, or both. They are detected by means of these rays either after the latter penetrate through the tissues or when a labeled compound has been isolated from the tissue under study. It should be recalled that Henry Becquerel (1896) accidentally discovered radioactivity when he observed that a fragment of uranium ore, a gamma-ray emitter, lying on a photographic plate, caused darkening of the plate emulsion. George Hevesy (1921) was the first to use a radioactive indicator in studying plant physiology. This was done by introducing a small amount of a natural radioactive isotope, Thorium B, into the plants under study. The way was paved for the use of radioactive tracers by Irene Curie and her husband, M. Joliot (1934), who were the first to induce radioactivity in normally stable chemical elements. Of the several hundred radioactive isotopes in existence today, those most frequently used in biological research include the radioactive isotopes of phosphorus, sodium, potassium, strontium, calcium, iodine, gold, iron, chromium, and carbon. Once these activated elements are incorporated into the biological system, they comprise minute sources of radiation, but otherwise behave identically like the normal element with regard to chemical properties. Isotopes, therefore, permit a study of the dynamic aspects of metabolism by their emitted radiation without interfering with normal function.

Mechanisms of radiation effects. An understanding of the mechanism of radiation effects on living material necessarily involves a knowledge of the basic phenomena that occur during the interaction of radiant energy with matter. The first consequence of such an interaction is the production of an ion pair. The mechanism may be pictured as follows: when radiation* is passed through matter, particular atoms distributed at random throughout the volume of the target material will absorb a quantum of radiant energy. This in turn will cause the atom to eject one of its orbital electrons at high speed from the atom, leaving the atom positively charged. The high-speed photoelectron thus ejected from the atom will travel a considerable distance through the matter before it loses its kinetic energy. When it has slowed down sufficiently it will become attached to another neutral atom that then becomes negatively charged, with the net result that a pair of oppositely charged ions is produced within the material. This process defines "ionization" as it applies to living matter. In a gas the process is similar except that the negative particles are mainly free electrons with comparatively few negative ions. Furthermore, the oppositely charged ions may, within

*Electromagnetic radiation (X rays and gamma rays). For corpuscular radiation, the interaction may be thought of as a direct collision of particles; the net effect, however, is the same as in the absorption of a quantum of electomagnetic energy.

a short time, neutralize each other by recombination owing to their Coulomb attraction. However, within a condensed system, such as a living cell, the oppositely charged ions can approach each other only by slow diffusion over a considerable distance, so that recombination may be inhibited for a protracted period of time. Thus the primary effect of the passage of ionizing radiation through living matter is the establishment of a certain concentration of ion pairs within the affected volume of material, which at any instant is in proportion to the intensity of the incident radiation.

If the atoms involved in the ionization process are in chemical combination with other elements, the ionization will have a profound effect on the chemical composition of the material. Since a chemical bond involves the transfer of an electron from one element to another, the ejection of such an electron from the atom in a compound will disrupt the chemical bond and cause decomposition of the molecule. The major consequence of the ionization, therefore, in a material such as the living cell, would be the fragmentation or decomposition of the normal cell constituents and the appearance of new chemical compounds. This, of course, can be expected to have a marked effect on the functioning of a biological system.

The exact sequence of events that causes the various abnormalities manifested in the irradiated living object is virtually impossible to ascertain exactly, since the loci of interaction are not accessible to direct observation. A number of theories have been proposed to explain the biological effects of radiation in terms of the fundamental processes of ionization and decomposition. Two of these, for which there has been some supporting experimental evidence, will be mentioned:

(1) *The target-hit theory.* This theory has been postulated by a number of investigators.[1-4] It explains the biological effect as the result of a direct hit of a quantum of radiant energy, a photon, upon a sensitive constituent in the cell. For example, the fragmentation of a chemical bond in a nuclear protein molecule following absorption of a photon would initiate a chain of events resulting in consequences deleterious to cellular function. The target theory has helped to explain the effects of radiation on unicellular organisms, such as viruses and bacteria, as well as on chromosome breaks and gene mutations in multicellular organisms. When biological entities such as eggs, bacteria, tissue cells, or whole animals are subjected to various doses of irradiation, a plot of the number of survivors versus the dose of irradiation yields an S-shaped curve, commonly known as a sigmoid curve. Sigmoid curves were obtained from studies on three different biological objects.[5] A study of these curves for many biological systems indicates that "multiple hits" are necessary in order to explain the over-all effect observed. In other words, all the vital constituents in a biological entity must be hit by radiation quanta. Most of the experiments designed to test the "target-

hit" theory have been carried out on single organisms, dry seedlings, or tissue particles so as to exclude the effects of the medium. I obtained typical sigmoid curves when tumor particles were subjected to various doses of X radiation prior to implantation into parent hosts.[6,7]

A number of phenomena induced by ionizing radiation cannot be explained adequately by the target-hit theory alone. These observations have led to the postulation of a second theory of radiation effects, namely, the free-radical theory.

(2) *Production of free radicals.* This theory, as postulated by Weiss,[8] assumes that the biological abnormalities result indirectly from the fragmentation of the more abundant cell constituents, such as water. In the latter case the photon, when absorbed by a water molecule, produces one pair of free radicals, namely hydrogen (H) and hydroxyl (OH). These free radicals, which are generated in a highly active state, diffuse throughout the cell and may be responsible for initiating abnormal chemical changes. Although the exact nature of these free radicals and their geometric distribution throughout the biological material are not known as yet, a study of their formation in simple systems, such as aqueous solutions, provides evidence for this theory. For the absorption of a photon by a water molecule, the following reaction has been generally accepted:

$$H_2O \longrightarrow H + OH$$

The free-radical theory is based on the idea that the primary ionization occurs predominantly in water molecules as the major cell constituent, and that the major secondary effect is the disruption of the water molecules into free radicals. The accumulation of monatomic hydrogen and of hydroxyl radicals within the material then initiates a chain of oxidation and reduction reactions in the irradiated region, causing the build-up of new chemical species that may be toxic to the cell. The extent of such radiobiological damage depends upon the distribution of the free radicals within the cell and the nature of their interaction with the cell constituents. A certain number of free H and OH radicals may, when sufficiently near each other, recombine to form a water molecule. However, it is plausible to postulate that an appreciable number will react with other cell constituents.

Of considerable interest is the case when excess oxygen is added to the system during irradiation. Under this circumstance the following reactions are likely to occur:

$$(1)\ H_2O \longrightarrow H + OH$$
$$(2)\ O_2 \longrightarrow 2\,O$$
$$(3)\ OH + O \longrightarrow HO_2$$
$$(4)\ HO_2 + H \longrightarrow H_2O_2$$

The formation of the hydroperoxyl radical HO_2, which is more stable than the hydroxyl radical OH, is considerably enhanced by the presence of free oxygen. In turn, the HO_2 radical can combine with monatomic hydrogen to form H_2O_2. In fact, the build-up of hydrogen peroxide in oxygenated aqueous media under irradiation is well established. The radiation damage may then be enhanced by HO_2 and H_2O_2, which are quite toxic to the cell life. As will be discussed later, the effect of oxygen in enhancing radiation damage supports this view.

It is further known that in simple aqueous systems radiation can produce oxidation and reduction reactions for example:

Ferrous ion oxidized to Ferric ion:

$$(Fe^{++} + OH \longrightarrow Fe^{+++} + OH^-)$$

Ceric ion reduced to cerous ion:

$$(C^{++++} + H \longrightarrow C^{+++} + H^+)$$

At present it cannot be stated with certainty whether either the target-hit theory or the free-radical theory is fully adequate to explain the biological changes observed in irradiated systems. The target-hit theory explains certain effects directly without the necessity of complicated intermediary processes. It does, however, seem to require a ratio of vital to nonvital volume within the cell that is in excess of the probable value in order to explain the magnitude of the observed effects. On the other hand, the free-radical theory, for which the generation of radicals can occur anywhere within the cell, seems plausible as far as quantitative, long-range effects are concerned, but it involves a complicated chain of events whose mechanism cannot be unraveled except by indirect observation of the resultant changes in the biological systems. It is probable that both mechanisms, physical and chemical, play a part in radiation effects. The extent to which one predominates over the other in any given biological system remains to be determined.

III. *Observations on Cell Dynamics Following Irradiation*

As mentioned in the introduction, the injurious effects on tissues induced by X rays and gamma rays were noted soon after their discovery by Roentgen and the Curies. Since the individual cells are the irreducible structural units of tissues, the study of radiation injury was naturally focused on cellular responses to the absorption of radiant energy. A vast amount of literature has accumulated on this subject over the past fifty years; the reader who is interested in this subject is referred to a number of books, monographs, and review articles dealing with this topic.[9-16] To illustrate the use of radiation as a tool in biological research, I have selected for discussion a few pertinent observations, particularly those pertaining to mammalian cells, both normal and ma-

lignant. The examples chosen for this discussion are by no means intended to be comprehensive, nor can they give adequate recognition to a large number of brilliant experiments that have contributed body and strength to the science of cellular radiobiology. These examples were chosen because of their relevance to the experimental work that has been carried out in my laboratory.

A difference in response to radiation among various types of tissues was one of the earliest observations in radiobiological research. As a result of these observations, two French scientists, Bergonie and Tribondeau[17] postulated the law that states that the biological effects of radiation vary directly with the reproductive ability of the cell and inversely with its degree of differentiation. Although this law generally holds true, particularly for normal embryonal tissues, it is not without exceptions. For example, the polymorphonuclear leukocyte is a differentiated cell, yet it is somewhat radiosensitive. On the other hand, macrophages that are regarded as primitive cells and spermatocytes, the majority of which are dividing cells, are quite radioresistant.[18] Conversely, the lymphocytes, nondividing cells, proved to be radiosensitive. Such exceptions are found also among malignant tumors. For example, I noted that a relatively undifferentiated mammary tumor proved to be more radioresistant when treated *in situ* than its analogue, which was relatively more differentiated.[19] For detailed accounts of variation in radiosensitivity among tissue cells the reader is referred to the findings of Desjardin[20] and Warren.[21] On the basis of the above-mentioned observations it may be inferred that there are factors other than rate of growth or differentiation that influence radiosensitivity.

The influence of radiation on the dynamics of cellular processes has long been recognized as a valuable tool for probing the complexities of cell function. The techniques developed for utilizing radiation in this area have been manifold. The tissue culture method and cinephotomicrography enable the investigator to note microscopically the effects of irradiation *in vitro* on the nuclear apparatus, that is, on the dynamics of cell division during various phases of its cycle, and on the cytoplasmic constituents.

Effects on nuclear apparatus. A delay in division after irradiation was one of the first effects observed on the dynamic properties of cells. This holds true for every type of cell studied. (The fact that the cell nucleus appeared to be most affected by irradiation of whole tissues was noted as early as 1911[22].) Depression of mitotic division may last several hours or several days, depending upon the radiation dosage. The temporary arrest of mitosis may permit the cells eventually to complete division and to produce apparently normal daughter cells. These phenomena were described by Strangeways and Oakley as early as 1923[23] and later by Spear and Glucksman.[24] The prolongation of the metaphase stage is a particularly characteristic feature of irradiated cells. For

example, such a phenomenon was observed in sections of tadpoles[25] and in a section of an irradiated tumor in which six cells in metaphase were noted in one microscopic field.[19] The injurious effect of ionizing radiation on the nuclear apparatus is also manifested by aberration and breakage of chromosomes. If the injuries are not too severe, the broken parts of the chromosomes may reunite and mitosis may proceed to completion.[26] This is regarded as a temporary or reversible effect. Other temporary effects are stickiness of chromosomes, delay in their movements,[27] and extension of the interphase.[28] Permanent or irreversible effects of irradiation are exemplified by fragmentation of chromosomes and coagulation of the chromatin substance, consequences which appear immediately after irradiation.

In evaluating radiation effects on nuclear apparatus, there is another important consideration, namely, the effects on the genes, resulting in mutation. It is noteworthy that this phenomenon may not become manifest in the first generation of the irradiated cells, but may appear in subsequent generations of the daughter cells. Thus the effect of radiation on the genes may be regarded as permanent. It is relevant to mention that Muller[29] first demonstrated that ionizing radiation greatly increases the frequency of mutation in *Drosophila*. This important finding was subsequently noted in irradiated plants,[30] in microorganisms,[31] and in mammals.[32] Chromosome aberrations or deletions were also produced in human cells, as was recently demonstrated by Bender.[33]

The actual phase of the mitotic cycle in which the cell is the most vulnerable to radiation injury has been a matter of dispute among various investigators. The most favored opinion is that the cell is most radiosensitive in the prophase when the chromatin substance is in the most concentrated form.[34-36] The relatively greater radiosensitivity of the lymphocytes is explained on this basis. The anaphase stage of the mitotic cycle is used chiefly as a criterion for the evaluation of radiation effects on a cellular level. Chromosomal "bridge" formation is a characteristic phenomenon in this phase of mitosis. Several examples of phenomena induced in the nuclear apparatus by X radiation are illustrated in microphotographs (FIGURES 1 to 3). FIGURES 1b and 3a present rather rare phenomena which, to my knowledge, have not been described before in mammalian cells.

Effects on the cytoplasm. The effects of radiation on cytoplasmic structures are not as apparent as the effects on the nucleus, and it is not quite clear which of the cytoplasmic components is the most radiosensitive. Ludford[37] noticed in mouse tumors that the earliest visible effects of radiation were on mitochondria that became vesicular and swollen 40 min. after irradiation, while inhibition of mitosis was not observed until 4½ hours later. The Golgi apparatus became apparent only 24 hours later, as compared to the 40-min. period for mitochondria. However, in further detailed studies by Ludford, as well as by others,[38]

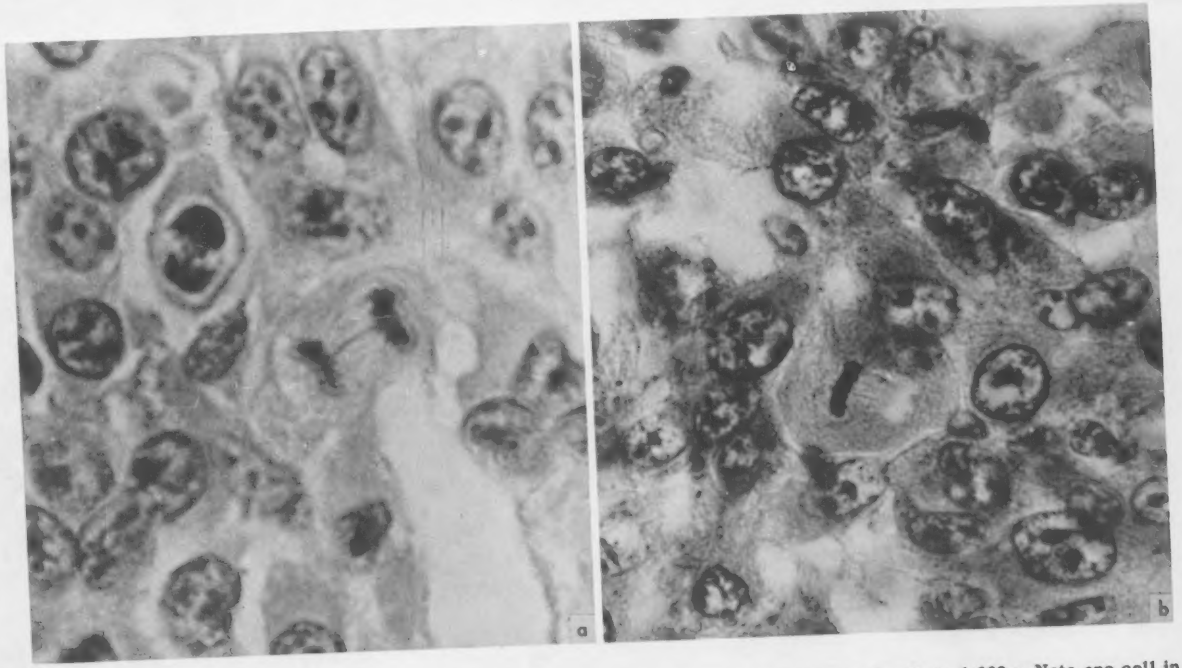

FIGURE 1. (a) A section of a DBAG tumor that was removed 72 hours after exposure *in situ* to 1,000 r. Note one cell in anaphase with a chromosome bridge and homogenous cytoplasm; the other cell, also in anaphase, with coagulated chromatin and intranuclear vacuolation. H + E X900. (b) A section of a DBAG tumor that was removed 48 hours after irradiation *in situ* with 1,000 r. Note the mitotic figure in anaphase with the chromosomes lined up in one pole, the cytoplasm being homogenous. H + E X900.

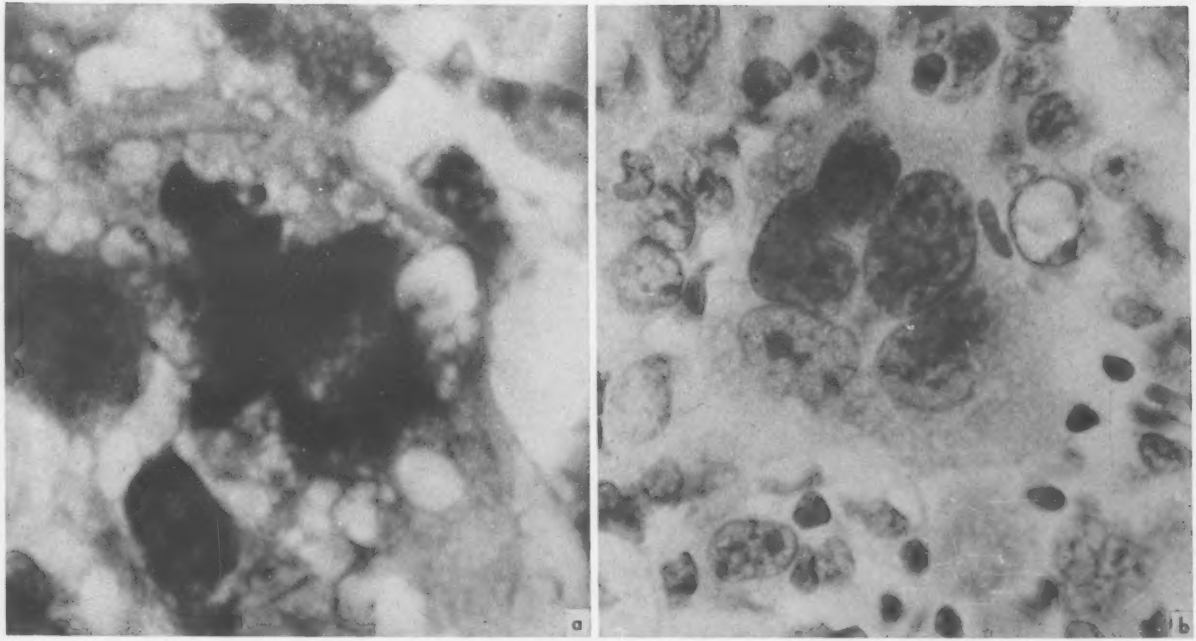

FIGURE 2. (a) A tripolar mitotic figure noted in a section of a fibrosarcoma growing in a rat. The section was removed from a tumor 96 hours after irradiation with 2,500 r. H + E X1500. (b) A section of the rat fibrosarcoma of the same type as in FIGURE 2a, which was removed 120 hours after exposure to 2,500 r. Note the cell with 5 nuclei. H + E X1500.

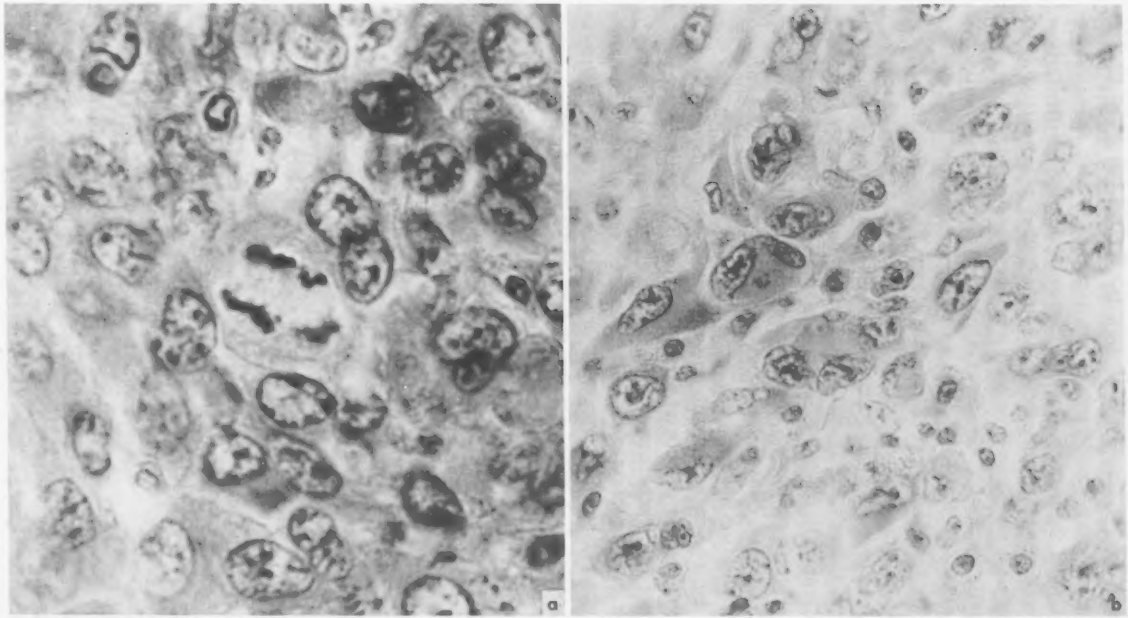

FIGURE 3. (a) A section of a DBAG tumor. This section was removed 144 hours after irradiation with 1,000 r. Note 2 anaphases in the same cell. H + E X900. (b) A section of a DBAG tumor. This tumor was treated *in situ* with a dose of 5,000 r under 2 atmospheres of oxygen pressure and removed from the animal host 7 days after radiation. Note the multinucleated cells, the cells with nuclei eccentrically located, and the dense cytoplasm. H + E X500. This figure is in contrast to FIGURE 4b, illustrating a section of a nonirradiated DBAG tumor.

it was found that the effects of radiation on mitochondria and Golgi apparatus became apparent only in lethally injured cells. Other cytoplasmic abnormalities manifested in irradiated cells are: vacuolization, liquefaction, and keratinization, the latter appearing particularly in squamous epithelium. The question as to whether the nucleus or the cytoplasm is most radiosensitive still remains unanswered. For example, Duryee[39] believes that the nuclear abnormalities noted in cells after irradiation are indirect and are produced by a constituent of the irradiated cytoplasm. On the other hand, Zirkle and Bloom,[40] who irradiated a cell nucleus with a very fine beam of protons, observed typical chromosome breaks, while no visible changes were seen when the proton beam passed near, but not through the nucleus, traversing only the cytoplasm. The opinion still prevails that the nucleus is the most radiosensitive part of the cell. Mitotic arrest and nuclear abnormalities that occur following irradiation thus far remain the most sensitive criteria for evaluating an effective radiation dosage.

Freshly excised fragments of animal tissue were also utilized for investigations on the effects of irradiation on live cells. Some of the advantages of this technique may be listed as follows: (1) the use of tissue particles or aggregates of cells simulates more closely the natural conditions when tissues are irradiated *in situ* than when loosely growing cells are irradiated *in vitro;* (2) the use of particles of organs (heart, kidneys, tongue, liver, and others that contain a variety of cell types) makes possible the detection of a selective response of the various cell types to a given radiation dosage; and (3) the irradiated tissue fragments of neoplastic origin can be cultivated *in vivo* by reimplanting them into parent hosts, thereby permitting observation of their response to radiation under normal environmental conditions and allowing also a comparison of the irradiation-induced changes within cells grown *in vitro* and those grown *in vivo.*

Studies with tissue fragments elicited a number of valuable observations. A difference in response to an equal dose of X radiation was noted among various types of cells within the same tissue particle. For example, when fragments of kidney tissue were irradiated and later cultivated *in vitro,* the lymphocytes, leukocytes, infiltrating cells, macrophages, and fibroblasts were gradually destroyed by increasing the radiation dosage, while the renal epithelial cells continued to proliferate.[41,42]

Studies on malignant, transplantable animal tumors demonstrated that mammary adenocarcinomas of very similar morphologic structure differed significantly with respect to the nuclear and cytoplasmic abnormalities induced by exposure of particles of both types of tumors to equal X-ray doses. Furthermore, the radiation doses required to prevent the tumor particles from growing *in vitro* were significantly greater than those required for preventing their growth *in situ.*[43]

To pinpoint the site of interaction of radiation energy with the biological system, the effects of radiation on specific metabolic processes were studied in my laboratory. In particular, studies were made of the effects of radiation on respiration, on aerobic and anaerobic glycolysis, and on the synthesis of nucleotides, such as adenylic acid, adenosine diphosphate, and adenosine triphosphate of both normal and malignant tissue cells. It was found that much larger doses were required to produce measurable effects on these processes than to prevent cell proliferation, *in vitro* as well as *in vivo*.[44,45] Furthermore, it was found that the tumor tissue possessing a relatively greater metabolic activity, as determined by the rate of incorporation of radiophosphorus (P^{32}) into the nucleotides, also possessed a relatively greater degree of recovery following medium or sublethal doses of radiation.[46]

Recent experiments on two different types of transplantable tumors, one epithelial and the other a spindle cell, both indigenous to the same host, have yielded some interesting observations regarding the relationship between radiosensitivity and metabolic as well as structural properties.[47] It was found that these tumors differ in their response to a given dose of radiation. Furthermore, the epithelial-type tumor exhibited a greater rate of anaerobic glycolysis as well as of oxidative phosphorylation than did the spindle-cell type tumor. A difference was also noted in the quality and integrity of the mitochondria of these two types of tumors. The above-mentioned properties might have some bearing on the difference in their response to radiation.

Numerous experiments have been carried out on a variety of biological systems to determine the effects of radiation on metabolic processes. It has been noted in general that the radiation dose required to produce an observable effect on any of the metabolic processes studied is larger than that required to affect cell division. For more details on this subject the reader is referred to a recently published monograph.[48]

IV. *Modification of Radiation Effects by Environmental Factors and Chemical Agents*

Modification of radiation response of biological systems by various agents and by environmental factors has been the subject of important investigations. Examples of such environmental factors and agents are the *p*H of the medium,[49,50] the temperature,[51,52] the phase (solid or liquid),[53,54] and aerobiosis and anaerobiosis. For detailed accounts of various factors influencing radiation effects, the reader is referred to a comprehensive review on this subject.[55]

One of the most promising phases of radiobiological research concerns the effect of various biological and chemical agents upon the response to radiation. Interesting results have been obtained in that agents that can decrease or increase the effects of radiation have been found. In

the former area sufficient effort has been made in recent years to make the appearance of practical applications seem imminent. Particular reference is made to the search for an agent to counteract radiation injury in mammals. As a consequence of the development of atomic weapons, the increased probability of exposure to total-body radiation has directed investigative effort to experiments involving whole organisms. Various biological and chemical agents have been and are currently being tested for their prophylactic effect on a variety of living objects, including bacteria, yeast, and mammals such as mice, rats, dogs, and swine. The current importance of this phase of the subject warrants mention of some details of recent accomplishments. Attention has been given to both biological and chemical means of protection against radiation injury.

Biological means. Since the blood-forming organs such as the spleen and bone marrow were shown to be the most radiosensitive, it was logical to devise methods to protect these organs or to find means by which to stimulate their activity or regeneration following radiation injury. Jacobson[56] was the first to demonstrate that, when the spleen was shielded during irradiation of the animal, its life span was significantly greater than that of an irradiated, nonshielded animal. Furthermore, the shielded animal could tolerate a significantly larger radiation dosage.

Injection of bone marrow[57] into animals receiving lethal doses of radiation was also effective in extending their life span by repopulating the depleted organism with fresh blood cells. Both of these methods have limitations in practical application. In the first place, protection of the spleen during total-body exposure presents a difficult physical problem in an emergency. With regard to bone marrow, only isologous material can be of value, since the bone marrow of genetically different individuals produces anaphylactic reactions due to production of antibodies. The isolation of a substance that might regenerate the blood-forming organs in lethally irradiated individuals would represent a more desirable objective. Attempts along this line have been made by several investigators,[58-60] who showed that various cell-free extracts of hematopoietic tissues exerted some protection. However, additional studies are required to prove the practical value of such extracts. The probability that cell-free preparations of the hematopoietic system might protect against radiation injury is of great interest for two reasons: (1) it might lead to a practical treatment of fatally irradiated human beings; and (2) it may prove that a humoral factor is involved in the protection of the reticuloendothelial system. Hormones[61-63] and vitamins[64,65] have also afforded some protection for irradiated animals.

Chemical agents. The possibility that chemicals may modify radiation effects stems from the recently developed theory that the biological effects of ionizing radiation are mediated by chemical reactions induced in irradiated, aqueous systems. Considerable attention has been given

to sulfhydryl substances as possible agents for counteracting radiation effects. This interest was stimulated by the observations that enzymes containing SH-groups are sensitive to oxidation when X-irradiated in aqueous solutions. The addition of sulfur-containing compounds appears to protect the enzymes and reduce the radiation effect.[66] It has been shown that a number of sulfur-containing compounds do, in fact, reduce radiobiological damage in mammals. Patt *et al.*[67] first showed that cystein reduced the radiation effects in lethally irradiated animals. Cysteinamine proved to be even more effective.[68] Other sulfur-containing chemicals were also effective in reducing radiation effects.[69-71] With regard to the protective mechanism of these preparations, the opinion is advanced that these chemicals act by removing the oxygen from the medium, thereby producing an anoxic state* in the treated organism.

It should be stressed, however, that all the preparations tested thus far have merely prolonged the survival of lethally irradiated animals for certain limits of time in comparison with irradiated, nontreated control animals. The majority of irradiated animals, treated or nontreated, eventually succumb as the result of the so-called "delayed" radiation effects, which are manifested in a variety of degenerative changes leading to sclerosis, malignant tumors, and premature aging. Thus, although important advances have been made, the search for an effective prophylactic agent must continue.

Conversely, a group of investigators has been devoting its time to finding an agent effective in increasing radiation damage. The motivation for this type of work, aside from its fundamental aspects, has been the possibility of increasing the effectiveness of radiation therapy in the treatment of malignancy. One of the most challenging subjects in this field is the problem of radiation effects under aerobic and anaerobic conditions. This topic is under study in a number of laboratories. I, too, have investigated the influence of aerobiosis and anaerobiosis upon the response of biological material to radiation. Since the findings represent a good example of the usefulness of radiation as a tool of biological research, the remainder of this paper will be devoted to a detailed description of our recent studies.

V. *A Study of Radiation Effects Under Aerobic and Anaerobic Conditions*

As early as 1909[72] it was observed that restriction of blood circulation, thereby limiting the oxygen supply, increased the resistance of human skin to irradiation. It was later noted that induction of anaerobic conditions may increase radioresistance of lower forms of life,[73] of normal mammalian objects,[74] and of tumor grafts.[75] Conversely, Mottram[76]

*This view correlates with the enhancing effect of oxygen in increasing the extent of radiation damage, as discussed below.

was the first to contend that aerobiosis increases the response to radiation. He based his opinion on the observation that bleeding of an animal prior to irradiation increased the radioresistance of the tumor. No data were then available to explain the effects of aerobiosis or anaerobiosis upon irradiated biological systems, and no practical value was attributed to these early observations.

In recent years, however, a new interest has developed in the influence of aerobiosis and anaerobiosis on radiobiological damage, stimulated by the concept of Weiss:[8] namely, that the various effects caused by ionizing radiation are due to chemical reactions mediated by activated free radicals in aqueous systems. As discussed in Section II, ionizing radiation splits the water molecule and produces activated free hydroxyl groups OH, as well as nascent hydrogen H, which are capable of inducing oxidizing and reducing reactions. In the presence of oxygen, the formation of oxidizing radicals HO_2 is enhanced, resulting in increased toxicity to the cell. It should be recalled that Risse[77] first made the suggestion that chemical processes are involved in irradiated aqueous systems and that Fricke *et al.*[78] first presented evidence that chemical reactions occur in such systems. Whereas the influence of oxygen[79-86] and of nitrogen[87-90] on the radiosensitivity of various normal biological objects has been studied extensively in recent years, relatively few attempts have been made to test the effects of increased oxygen concentration on the response of experimental tumors to X radiation.[91,92] The effect of oxygen upon tumors irradiated with X rays *in vitro* has not been studied extensively. To my knowledge, only one reference pertaining to ascites tumor cells is available.[93] For this reason, and in view of the importance of this type of investigation, I undertook experiments on sections of solid tumors in order to determine to what extent oxygen or nitrogen may influence their response to X radiation.

Material and methods. The *in vitro* irradiation method was chosen for this initial study since this technique permits carrying out experiments under controlled environmental conditions.

The biological material chosen for this study consisted of two mouse mammary tumors, designated respectively as DBAG and $DBAH_1$, and rat mammary tumor designated Ar. All three of these tumors are indigenous to inbred hosts and thus represent genetically homogeneous material. For further details on these tumors see FIGURE 4.

For each experiment one or two of each tumor type was excised from the parent host under aseptic conditions. Slices about 0.3 mm. thick were cut from the edges of each tumor and immediately placed into sterile mammalian Ringer's solution at *p*H 7.4 and kept refrigerated until exposed to radiation. Tissue slices about 0.3 mm. thick permit complete gas diffusion through the cell.[94] Portions of the tumor slices were suspended free in the well of the newly devised plastic chamber (FIGURE 5*A*)

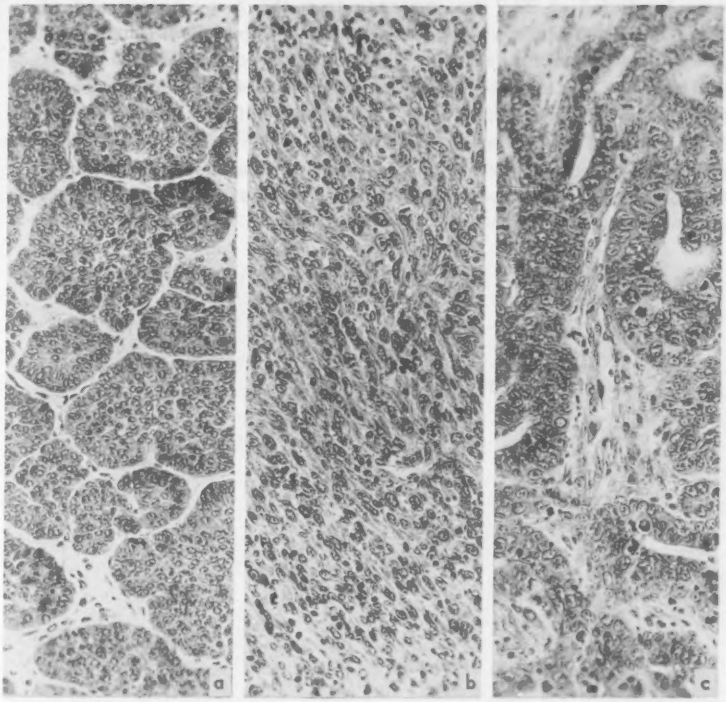

FIGURE 4. (a) Mammary adenocarcinoma ($DBAH_1$) indigenous to DBA/2 strain of mice. Note the nests of closely packed cells, mitoses, and the stroma surrounding the cell nests. H + E X300. (b) Spindle-cell mammary tumor (DBAG), also indigenous to DBA/2 strain of mice. Note the bundles of loosely connected spindle cells and mitoses. No apparent stroma. H + E X300. (c) Mammary carcinoma (Ar) indigenous to August strain of rats. Note the alveoli of closely packed cells, mitoses, and the stroma. H + E X300.

which contained approximately 10 cc. of ice-cold mammalian Ringer's solution. The gas to be used (air, or oxygen, or nitrogen) was bubbled through this well. The gas pressure from the tank was regulated so that the flow rate through the purification apparatus and through the tissue suspension in the plastic chamber was 1 l./min. The suspended tumor sections were gassed for 3 min. before irradiation and during radiation with a given X-ray dosage. FIGURE 6 illustrates the radiation set-up. Radiation and gassing were conducted at room temperature varying between 22 to 24° C.

For radiation purposes, a General Electric Maxitron, operating at 200 kvP. and 30 ma., was used. The X-ray beam was filtered by 0.5 mm. Cu plus 1 mm. Al., resulting in a HVL = 1.0 mm. Cu. The tumor sections

were irradiated at 22.2 cm. distance from the X-ray source and at an intensity of 400 r/min. In each experiment, 2 experimental and 2 control groups were treated as follows: (1) tumor sections X-irradiated in oxygenated medium; (2) tumor sections X-irradiated in aerated medium; (3) tumor sections oxygenated; and (4) tumor sections aerated. The tumor sections of series 3 and 4 were oxygenated and aerated in Ringer's solution for the same time as required for a given X-ray dose. These last 2 series served as controls. The same procedure was followed in the experiment with nitrogen.

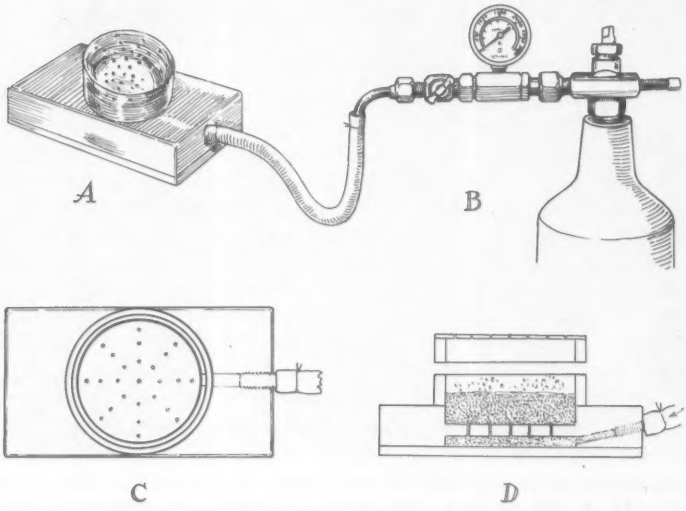

FIGURE 5. Plastic chamber. Views *A* and *B* illustrate the complete setup of the plastic chamber that is connected with the pressure gauge and oxygen tank by means of rubber tubing. The lucite block is 6 mm. long, 1.5 cm. thick, and 5 cm. wide, and was machined to receive a circular cavity measuring 4 cm. in diameter and 0.5 cm. in depth. Twenty-five perforations were made in this circular cavity to connect it with the lower reservoir to provide access to oxygen. A plastic ring 1.0 cm. high was cemented above the circular cavity, forming a well in which the tumor sections were suspended in Ringer's solution (*C* and *D*). A metal nipple inserted into one side of the plastic block leads into the lower reservoir and serves as a connection, by rubber tubing, with the oxygen supply. A circular perforated cover is provided to protect the tissue from contamination and to allow the excess oxygen to escape.

After irradiation the tumor sections were immediately transferred from the plastic chamber into small Petri dishes containing fresh, ice-cold Ringer's solution and from there were transplanted into the animals. Ten to twelve tumor grafts were used for each treated group of tissues. All of these manipulations were conducted under strictly aseptic conditions, effectively preventing contamination of the tumors. Male animals were used throughout these experiments.

An equal number of the experimental and control animals were kept in each stainless steel storage cage and were fed Purina Lab Chow and water *ad libitum*. The animals were inspected daily or every second day for tumor growth. Measurements in three dimensions were made of the growing tumors with Vernier calipers every second or third day. The tumors were allowed to grow until death of the animals. The time designated as the latent period (growth delay) and the number of positive "takes" served as criteria for evaluating the results of each experiment.

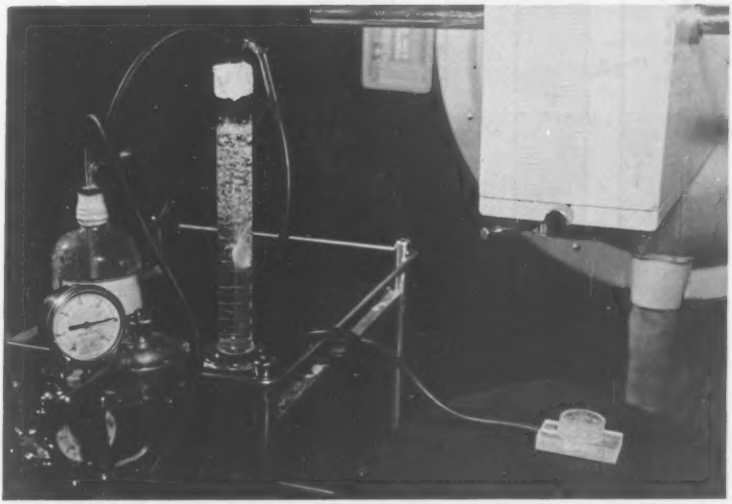

FIGURE 6. Radiation and gassing setup. Maxitron 250 kv. X-ray machine. Plastic chamber containing the suspended tumor sections in Ringer's solution. On the metal table is a bottle filled with concentrated barium hydroxide. It is connected to a glass cylinder filled with water and to the gas tank. Another connection leads from the glass cylinder to the plastic chamber.

Results

Observations on the DBAG tumor. The results obtained with this tumor are illustrated in FIGURE 7, from which it is evident that equal doses of X rays exerted a greater effect on the tumor sections that were irradiated in oxygen than on those irradiated in air. The nonirradiated tumor slices and tumor slices irradiated in air with doses of 2,000 r and 3,000 r produced tumors in 100 per cent of the grafted mice; those irradiated with doses of 2,000 r and 3,000 r in oxygenated medium produced tumors in only 50 and 25 per cent, respectively, of the grafted mice after prolonged latent periods.

Among the controls the latent period ranged from 7 to 12 days. When irradiated with 2,000 r, the latent periods of the tumor implants irradiated

in air ranged from 8 to 18 days, while those irradiated in oxygen ranged from 17 to 29 days. The relatively longer latent periods of the tumor implants irradiated in oxygen as compared with those irradiated in air were also noticed in the subsequent experiments on the two other types of tumors. The significance of this observation will be discussed later.

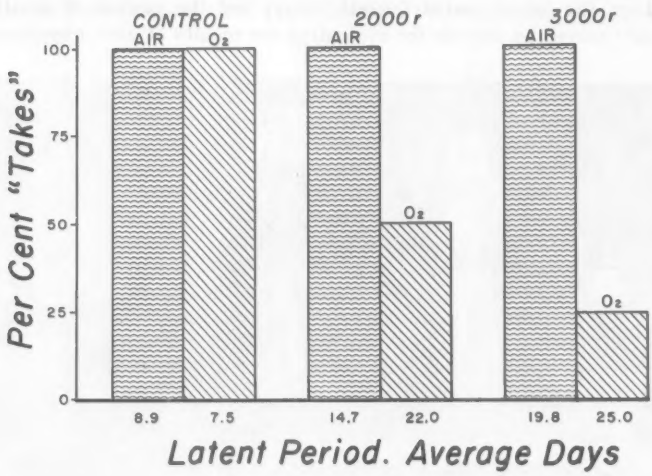

FIGURE 7. Results of the spindle cell (DBAG) tumor irradiated in air or in oxygen.

A dose of approximately 5,800 r in air, applied under standard* conditions, prevented the DBAG tumor grafts from proliferating, while a dose of 3,500 r achieved the same effect if irradiation took place in an oxygenated medium. Taking 5,800 r as a lethal dose in air and 3,500 r as a lethal dose in an oxygenated medium, one may conclude that the radiobiological response of the DBAG tumor grafts is increased by a factor of approximately 1.6 as a result of oxygenation.

Observations on the DBAH$_1$ tumor. In this series of experiments a dose of 2,500 r was used. This constitutes approximately 50 per cent of the lethal dose (LD_{50}) for grafts of this tumor under standard conditions*. The tissue sections were suspended in Ringer's solution through which either pure oxygen, or air, or a mixture of 40 per cent oxygen and 60 per cent nitrogen was bubbled for 3 min. before and during irradiation. Control tumor slices were similarly gassed but not irradiated. The results illustrated in FIGURE 8 show, that while the tumor grafts irradiated with 2,500 r in air produced tumors in 66 per cent of the implanted mice,

*Standard conditions indicate that freshly excised small tumor particles were placed on a round glass cover slip and irradiated under asceptic conditions.[13]

those irradiated in a mixture of 40 per cent O_2 and 60 per cent nitrogen produced tumors in only 50 per cent of the animals, and no tumors appeared from implants irradiated in an oxygenated medium, against 100 per cent "takes" of nonirradiated tumor grafts.

A dose of 5,300 r in air, applied *in vitro* under standard conditions* prevented the $DBAH_1$ tumor implants from growing in the grafted parent hosts. Taking 5,300 r as the lethal dose in air and 2,500 r as the lethal dose in oxygen, the radiobiological effect on the $DBAH_1$ tumor was increased by a factor of about 2.1, due to the oxygenation.

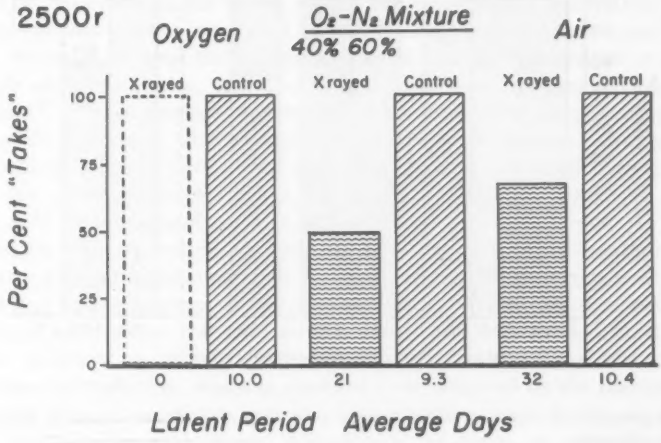

FIGURE 8. Results of the mammary adenocarcinoma ($DBAH_1$) irradiated in air, in oxygen, and in a mixture of 40 per cent oxygen and 60 per cent nitrogen.

Observations on the Ar tumor. Since the Ar tumor is highly radioresistant, the lethal dose being 6,500 r under standard conditions, three X-ray dose levels were employed and the influence of air, oxygen, and nitrogen was compared for each dose level. The results are illustrated in FIGURE 9. It can be seen that a dose of 1,000 r either in air or oxygen permitted the tumor grafts to produce tumors in 100 per cent of the parent hosts, the only difference being that the average latent period of the oxygenated irradiated implants was twice as long as that of the implants irradiated with an equal dose in air. Tumor grafts irradiated with 3,000 r in air produced tumors in 100 per cent of the hosts, while those irradiated in oxygen produced none. A dose of 4,000 r in air permitted 80 per cent of the grafts to produce tumors after an average latent period of 54 days,

*Standard conditions indicate that freshly excised small tumor particles were placed on a round glass cover slip and irradiated under asceptic conditions.[13]

while those irradiated with an equal dose in pure nitrogen produced tumors in 100 per cent of the parent hosts. None of the tumor grafts irradiated with 4,000 r in pure oxygen produced positive "takes". The protective effect of nitrogen in contrast to the increased injurious effect of oxygen is evident. Considering 6,500 r as the lethal dose in air and 3,000 r as the lethal dose in oxygen, the oxygen increased the radiobiological effect on this tumor by a factor of approximately 2.2.

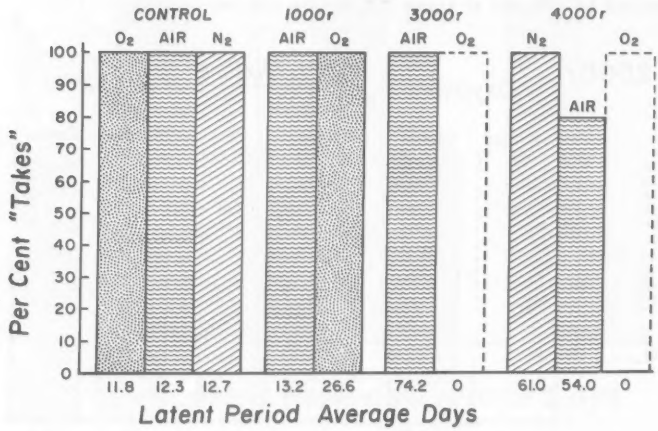

FIGURE 9. Results of the rat mammary tumor (Ar) irradiated in air, in oxygen, or in nitrogen.

Discussion of results. The above results show that sections of the three types of tumors irradiated in oxygen are affected more significantly than when irradiated in air or nitrogen. Dose-reducing factors (DRF) approximating 1.6 were obtained for the DBAG tumor, 2.1 for the DBAH tumor, and 2.2 for the Ar tumor. These factors are within the range obtained by other investigators (1.c.93). We may speculate on the difference between the DRF of 1.6 for the DBAG tumor and the DRF's for the DBAH and the Ar tumors that are very similar (2.1 and 2.2). The DBAG tumor (FIGURE 4b) is relatively more vascular and the cells are more loosely distributed than the $DBAH_1$ and Ar tumors (FIGURES 4a, 4c), which consist of nests of closely packed cells having a scanty blood supply. It may be assumed that the cells of the latter two tumors are therefore normally under relatively more anoxic conditions than the loosely distributed cells of the DBAG tumor. The potentiating effect of irradiation by oxygen is then greater on the more anoxic cells.

In histological sections of tumors irradiated *in situ,* nests of cells are often noted in some areas which do not exhibit any apparent radiation

injury, while in other areas the cells are destroyed. This uneven response of the tumor cells has been thought to result from a possible uneven distribution of the radiant energy within the tumor. However, from a purely physical point of view tumors about 1.0 cm. in diameter (irradiated with the physical factors such as those that I used) would be subjected to an even distribution of radiation within the tumor. On the basis of the present experiments it seems more likely that these radioresistant areas were remote from the blood supply, thereby being relatively more anoxic. The role that aerobiosis and anaerobiosis may play in radiation effects on experimental tumors was also brought forth by Hall *et al.*[95]

The extension of the latent period due to oxidative irradiation may indicate that greater impairment took place in the reproductive apparatus of the cells exposed to O_2 than in that of the cells irradiated in air. Such an interpretation would be in agreement with the observations of others[96,97] that chromosomes broken in the absence of oxygen are more capable of rejoining than chromosomes broken in the presence of oxygen. Should this interpretation hold true, the latent period might correspond to the period of mitotic arrest and would indicate the length of time required for recovery of partially injured cells.

If the primary action of ionizing radiation in aqueous systems is ascribed to the production of free hydroxyl radicals OH (oxidizing entities) and free hydrogen atoms H (reducing entities), the interaction of radiant energy with matter can then be based on chemical reaction kinetics. The potentiating effect of oxygen in the response of the biological system to irradiation, which is assumed to be mediated by the production of the strongly oxidizing radicals OH and HO_2, lends further support to the radiochemical theory. Furthermore, oxidizing or reducing free radicals may produce new substances that might be injurious to the life processes of the cell. However, it is not yet definitely known whether the primary injury to the cell is exerted by the free radicals *per se* or by new intermediate products formed by oxidizing or reducing processes in the medium during irradiation. If the effect is due to intermediate products formed by oxidizing or reducing radicals, oxygen would then exert its biological effect indirectly. Ionizing radiation may also produce biological damage by direct interaction as postulated by the target-hit theory described in Section II. It seems probable that both physical and chemical events are operative in producing radiobiological changes.

Another question that remains to be answered is whether the primary damage that leads to cell death is initiated in the nucleus or in the cytoplasm, as discussed in Section III. In experiments now in progress in my laboratory, slices of tumors irradiated with doses of X rays sufficient to prevent growth did not differ in the rates of respiration or of aerobic or anaerobic glycolysis when irradiated either in air or in pure oxygen. These observations indicate that the oxygen exerts a preferential effect on mitotic division rather than on metabolic activity of the cell when

respiratory and glycolytic enzyme activities are specifically involved. Observations that chromosomal damage is the major effect produced in plants and insects[96,97] irradiated in the presence of oxygen supports this view.

Summary

To emphasize the usefulness of ionizing radiation as a research tool in biology, and in order to illustrate several interesting areas for application of this tool, the material selected for presentation was divided into the following main categories:

Ionizing Radiations and their Action on Biological Systems

A discussion of the important physical properties of ionizing radiation was presented along with a description of the mechanism(s) of interaction of radiation with living material. The primary effect of radiation was described as the creation of ion pairs within the irradiated material, leading to the disruption of chemical bonds and the fragmentation of the constituent chemical compounds. Concerning the specific effects on living matter, two theories were presented, namely: (1) the target-hit theory; and (2) the free-radical theory. In the former theory, the radiation-induced decomposition occurs in a vital cell constituent following the fortuitous absorption of a quantum of radiant energy (photon) by a cell molecule in a particularly sensitive location. In the second theory the biological effect is attributed to a chain of oxidation and reduction reactions initiated by the generation of free radicals, which in turn results from the radiation-induced fragmentation of the water molecule. The latter process is assumed to take place throughout the cell.

Observations on Cell Dynamics Following Irradiation

In this section, the changes induced by radiation in cell structures and in dynamic cell behavior were considered. In particular, the prolongation of the normal mitotic cycle, chromosome aberration, mutation, and other effects on the nuclear apparatus were discussed. Effects on the cytoplasm, including enlargement of the mitochondria and fragmentation of the Golgi apparatus, were described.

Changes in cell function were described in terms of observations on tissue fragments, either cultured *in vitro* or implanted into an animal host following irradiation. Striking differences in radiosensitivity were noted among various cell types, even between cells of very similar morphological structure. Of particular interest with regard to cell metabolism was the observation that much larger doses are required to affect any of the metabolic processes studied than to affect growth.

Modification of Radiation Effects by Environmental Factors and Chemical Agents

The attempts to modify radiation effects in living systems by various agents were discussed in terms of the desirability of decreasing or increasing the response to radiation. In the first category efforts have been made to discover protective measures against radiation injury. Biological agents such as bone marrow and spleen preparations, hormones, and vitamins have afforded some protection. Similarly, certain chemical agents, particularly sulfur-containing compounds, also appeared to mitigate the extent of radiation damage in biological systems. In the second category, the potentiating effect of radiation by oxygen was discussed in detail, including the most recent work carried out in my laboratory. The increased effect of X radiation by oxygen on the tumors investigated was clearly demonstrated.

Many avenues of research have been discussed only briefly in this report. It was not possible to exhaust the many ramifications of past work, nor the multitude of implications for the future. Much fundamental work remains to be done in the broad field of radiobiology. One of the most important problems requiring further investigation involves recovery from radiation injury. These studies are needed to clarify such phenomena as the difference in radiosensitivity between various cell types. Such differences appear to be intimately associated with differences in recovery from radiation effects. An understanding of these processes may constitute an important step in elucidating the mechanism(s) involved in mutation, carcinogenesis, and aging induced by ionizing radiation.

It should be emphasized again that in the present atomic era research on the effects of ionizing radiation on biological systems occupies an important position in the biological sciences. Of greatest significance, perhaps, is the promise the study of radiobiology holds in the relief of human suffering by way of radiation therapy and prophylaxis.

Acknowledgments

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CONFERENCES HELD

SECTION OF BIOLOGY

HEMATOPOIETIC MECHANISMS

Thursday, May 1 and
Friday, May 2, 1958

Conference Co-Chairmen

Albert S. Gordon
Department of Biology, New York University, New York, N. Y.

Walter S. Root
College of Physicians and Surgeons, Columbia University, New York, N. Y.

THURSDAY, MAY 1, 1958

SESSION I

TECHNIQUES USED IN THE QUANTITATIVE STUDY OF BLOOD CELLS

Session Chairman: Maxwell M. Wintrobe
University of Utah College of Medicine, Salt Lake City, Utah.

9:00 A. M.—

Greetings from the Academy — Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

"Quantitative Aspects of Erythropoiesis"—Clement A. Finch, University of Washington School of Medicine, Seattle, Wash.

"Granulocyte Production and Destruction in Normal and Leukemic Subjects"—Howard R. Bierman, City of Hope Medical Center, Duarte, Calif.

"Cross-Circulation Experiments in Elucidating the Viability and Distribution of Leukocytes" — Stuart C. Finch, Yale University School of Medicine, New Haven, Conn.

"Regulation of Leukocytic Levels" — Clara M. Ambrus and Julian L. Ambrus, Roswell Park Memorial Institute, Buffalo, N. Y.

"Tissue Culture of Bone Marrow"—Edward H. Reisner, St. Luke's Hospital, New York, N. Y.

SESSION II

HORMONE CONTROL OF BLOOD CELL FORMATION

Session Chairman: Walter S. Root

College of Physicians and Surgeons, Columbia University, New York, N. Y.

2:00 P. M.—

"Oxygen Need as a Factor in the Anemia of Hypophysectomized Animals"—Roger C. Crafts and Howard A. Meineke, University of Cincinnati College of Medicine, Cincinnati, Ohio.

"Influence of Hypoxic Stimuli Upon Blood Formation in Endocrine-Deficient Animals"—Sam J. Piliero, New York Medical College, New York, N. Y.

"The Pituitary Erythropoietic Factor"—Donald C. Van Dyke, Donner Laboratory, University of California, Berkeley, Calif.

"Studies on the Control of Erythropoiesis"—Leon O. Jacobson, University of Chicago, Chicago, Ill.

"Some New Experimental Data Concerning the Role of the Adrenals in the Regulation of Blood Eosinophilia"—A. Aschkenasy, Hôpital de la Pitié, Paris, France.

SESSION III

Session Chairman: C. J. Watson

University of Minnesota Medical School, Minneapolis, Minn.

8:00 P. M.—

"Hemolytic Mechanisms"—William Dameshek, New England Center Hospital, Boston, Mass.

Discussion: William H. Crosby, Jr., Walter Reed Army Medical Center, Washington, D. C.

FRIDAY, MAY 2, 1958

SESSION IV

HEMOPOIETINE

Session Chairman: Wilson C. Grant

Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

9:00 A. M.—

"Erythropoietic Factor in the Control of Red Cell Production"—Allan J. Erslev, Boston City Hospital, Boston, Mass.

"Some Observations on the Stimulation of Erythropoiesis by Humoral Factors"—James W. Linman, Northwestern University Medical School, Chicago, Ill., and Frank H. Bethell, Thomas Henry Simpson Memorial Institute, Ann Arbor, Mich.

"Studies on the Actions and Properties of the Circulating Erythropoietic Stimulating Factor" – Albert S. Gordon, New York University, New York, N. Y.

"Current Studies on the Influence of Erythropoietine on Erythropoiesis" – Edwin A. Mirand, Roswell Park Memorial Institute, Buffalo, N. Y.

"Effects of Hematopoietine on Iron Metabolism in Normal and Starved Animals: Studies with Fe^{59}" – George Hodgson, University of Chile, Santiago, Chile.

"Present Status of the Physiology of Erythropoietine" – Frederick Stohlman, Jr., National Institutes of Health, Public Health Service, Bethesda, Md.

Discussion: Henry Borsook, California Institute of Technology, Pasadena, Calif.

SESSION V

LIFE SPAN OF BLOOD CELLS

Session Chairman: Irving London

Albert Einstein College of Medicine, New York, N. Y.

2:00 P. M. –

"Studies of Red Cell Survival" – George S. Eadie, Duke University School of Medicine, Durham, N. C.

"Radioautographic Studies of Iron Kinetics" – Edward L. Alpen, U. S. Naval Radiological Defense Laboratory, San Francisco, Calif.

"Patterns of Granulocyte Distribution and Regulation" – Harvey M. Patt, Argonne National Laboratory, Lemont, Ill.

"A Method for Labeling Leukocytes with Radioactive Diisopropyl Fluorophosphate (DFP^{32})" – J. W. Athens, A. M. Mauer, Helen Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe, University of Utah College of Medicine, Salt Lake City, Utah.

"Blood Cell Survival in Tissue Culture" – Edwin E. Osgood, University of Oregon Medical School, Portland, Oreg.

SESSION VI

ROUND TABLE DISCUSSION

8:00 P. M. –

Academy Building

The Section of Biology provides conferences for active workers in the special fields of biology.

Attendance is limited to those invited to participate in these conferences and to interested Members of the Academy.

PSORIASIS

Friday, May 9, 1958

Conference Chairman: Peter Flesch

Department of Dermatology, University of Pennsylvania, Philadelphia, Pa.

FRIDAY, MAY 9, 1958

Session Chairman: Carl T. Nelson

College of Physicians and Surgeons, Columbia University, New York, N. Y.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

"Clinical Features of Psoriasis" – George W. Hambrick, Jr., Department of Dermatology, University of Pennsylvania, Philadelphia, Pa.

"Pathology of Psoriasis" – Elson B. Helwig, Dermal and Gastro-Intestinal Section, Armed Forces Institute of Pathology, Washington, D. C.

"Recent Histochemical Findings in Psoriasis" – Otto Braun-Falco, Universitäts-Hautklinik, Mainz, Germany.

"The Application of Paper Electrophoresis to the Diagnosis of Psoriasis" – Daphne Anderson Roe, Vassar College, Poughkeepsie, N. Y.

"Chemical Changes in Psoriatic Scales" – Peter Flesch, Department of Dermatology, University of Pennsylvania, Philadelphia, Pa.

Session Chairman: Peter Flesch

Department of Dermatology, University of Pennsylvania, Philadelphia, Pa.

2:00 P.M. –

"Observations on the Problem of Pathogenesis in Psoriasis" – Allan L. Lorincz, Section of Dermatology, Department of Medicine, University of Chicago, Chicago, Ill.

"Possible Significance of Elevated Arginase Activity in Psoriasis Scales" – Simon Rothberg, Dermatology Service, National Cancer Institute, Public Health Service, Bethesda, Md.

"Psoriatic Arthritis" – Alfred Jay Bollet, Rheumatic Disease Unit, Department of Medicine, Wayne State University College of Medicine, Detroit, Mich.

"Therapeutic Approaches in Psoriasis"—M. Harris Samitz, Department of Dermatology, University of Pennsylvania, Philadelphia, Pa.

"Clinical Experience With a New Preparation for the Treatment of Psoriasis"—Jacob Bleiberg, Beth Israel Hospital, Newark, N. J.

"Clinical Experience with a New Preparation for the Treatment of Psoriasis: A Paired Comparative Study"—Sidney G. Clyman, Skin and Cancer Unit, University Hospital, New York University-Bellevue Medical Center, New York, N. Y.

GERMFREE VERTEBRATES: PRESENT STATUS

Thursday, May 15 and
Friday, May 16, 1958

Conference Chairman: James A. Reyniers

Lobund Institute, University of Notre Dame
Notre Dame, Ind.

THURSDAY, MAY 15, 1958

INSTRUMENTATION FOR REARING GERMFREE VERTEBRATES

Session Chairman: M. J. Kopac

Department of Biology, New York University
New York, N. Y.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

Introduction to the Conference – James A. Reyniers, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"The Gustafsson Apparatus" – Bengt Gustafsson, Department of Germfree Research, Institute of Histology, University of Lund, Lund, Sweden.

"The Use of Plastics in the Design of Isolator Systems" – Philip C. Trexler, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Remote Control Apparatus of Miyakawa" – Masasumi Miyakawa, Department of Pathology, Nagoya University School of Medicine, Nagoya, Japan.

"The Reyniers Germfree System II" – James A. Reyniers, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Housing of Disease-Free Vertebrates" – Henry L. Foster, Charles River Breeding Laboratories, Inc., Brookline, Mass.

REARING GERMFREE VERTEBRATES

Session Chairman: Sidney Weinhouse

Institute for Cancer Research and Lankenau Hospital
Philadelphia, Pa.

2:00 P.M. –

Introduction – "Nutrition and Biochemistry of Germfree Vertebrates: General and Theoretical Aspects" – Sidney Weinhouse, Institute for Cancer Research and Lankenau Hospital, Philadelphia, Pa.

"Determination of Germfree Status" – Morris Wagner, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Theoretical and Practical Considerations of Germfree Animals in Virology" – Alton R. Taylor, Virus Research Division, Parke, Davis & Company, Detroit, Mich.

"Rearing Germfree Cesarean-Born Rats, Mice, and Rabbits Through Weaning" – Julian R. Pleasants, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"The Nutrition and Biochemistry of Germfree Chicks" – Thomas D. Luckey, Department of Biochemistry, University of Missouri, Columbia, Mo.

"Vitamin K Efficiency in Germfree Rats" – Bengt Gustafsson, Department of Germfree Research, Institute of Histology, University of Lund, Lund, Sweden.

"Nutrition of Germfree Mammals" – Bernard S. J. Wostmann, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Studies on Rearing the Guinea Pig Germfree" – Bruce P. Phillips, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md.; Patricia A. Wolfe and Helmut A. Gordon, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

FRIDAY, MAY 16, 1958

CHARACTERISTICS OF GERMFREE AND DISEASE-FREE ANIMALS

Session Chairman: Paul György

Clinic of Pediatrics, Philadelphia General Hospital
Philadelphia, Pa.

9:00 A.M. –

"Morphologic and Physiological Characteristics of Germfree Animals" – Helmut A. Gordon, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Lymphatic System of Germfree Guinea Pigs" – Masasumi Miyakawa, Department of Pathology, Nagoya University School of Medicine, Nagoya, Japan.

"Some Histological and Functional Aspects of Lymphoid Tissues in Germfree Animals" – G. J. Thorbecke, Department of Pathology, New York University-Bellevue Medical Center, New York, N. Y.

"Serum Proteins in Germfree Vertebrates" – Bernard S. J. Wostmann, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Serologic Aspects of Germfree Life" – Morris Wagner, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Disease-Free Laboratory Animals as Related to Germfree Life" – Robert D. Henthorne, Microbiological Associates, Bethesda, Md.

"Disease in Disease-Free Animals" – James R. N. Innes, Burroughs Wellcome & Co., Wellcome Research Laboratories, Tuckahoe, N. Y.

USE OF GERMFREE VERTEBRATES IN RESEARCH

Session Chairman: Gosta Glimstedt

Institute of Histology, University of Lund
Lund, Sweden

2:00 P.M. –

"The Germfree Animal as a Research Tool" – Gosta Glimstedt, Institute of Histology, University of Lund, Lund, Sweden.

"A Review of Dental Research Using Germfree Animals" – Francis Orland, Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.

"A Comparison of the Development of Some Rat and Mouse Helminths in Germfree and Conventional Guinea Pigs" – Walter L. Newton, Paul P. Weinstein, and Myrna F. Jones, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md.

"The Use of Germfree Guinea Pigs in Studies on the Microbial Interrelationships in Amoebiasis" – Bruce P. Phillips, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md., and Patricia A. Wolfe, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

"Hemorrhagic Shock and Germfree Life" – Benjamin W. Zweifach, Department of Pathology, New York University-Bellevue Medical Center, New York, N. Y.

"Growth Response and Effect on Intestinal Flora in Germfree and Conventional Animals Fed Antibiotics" – Martin J. Forbes, J. Y. Parks, and M. Lev, Germfree Life Laboratory, Walter Reed Army Medical Center, Washington, D. C.

"Tumorgenesis and the Germfree Chicken" – James A. Reyniers and Miriam R. Sacksteder, Lobund Institute, University of Notre Dame, Notre Dame, Ind.

PANEL DISCUSSION

PRESENT STATUS AND FUTURE DEVELOPMENT OF GERMFREE LIFE STUDIES

Panel Chairman: Willard H. Wright
Laboratory of Tropical Diseases
National Institute of Allergy and Infectious Diseases
Public Health Service, Bethesda, Md.

8:00 P.M. –

Introductory Remarks – Willard H. Wright, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md.

"Infectious Diseases, the Pure Culture Concept and Germfree Life" –

"Allergy, Immunity and the Germfree Animal" –

"Host-Contaminant Relationships: The Activation of Immunity Systems, the Problems of Intestinal Synthesis and Aging" –

"Germfree Life and the Metabolic Diseases" –

"The Problem of Apparatus, the Supply of Germfree Animals, and the Need for Standardizing the Techniques and Animals" –

"The Need for an Adequate Terminology" –

"The Knowledge Necessary in the Development, Anatomy, Biological Characteristics, and Physiology of Germfree Animals Before Their Use in Experimental Research" –

"The Germfree Animal and Techniques as Tools in Cancer Research" –

THE BASIC AND CLINICAL RESEARCH OF THE NEW ANTIBIOTIC, KANAMYCIN

Thursday, July 10, 1958
Friday, July 11, 1958

Conference Chairman
Maxwell Finland
Thorndike Memorial Laboratory, Boston, Mass.

THURSDAY, JULY 10, 1958

Session Chairman: Henry Welch
Division of Antibiotics, U. S. Food and Drug Administration, Washington, D. C.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

Conference Opening and Introduction of Conference Chairman – C. H. Mann, Bristol Laboratories Inc., New York, N. Y.

Statement of Conference Purpose – Maxwell Finland, Thorndike Memorial Laboratory, Boston, Mass.

Introduction of Guests from Japan – A. R. Menotti, Bristol Laboratories Inc., Syracuse, N. Y.

"Kanamycin: Its Discovery" – Hamao Umezawa, Antibiotic Division, National Institute of Health, Tokyo, Japan.

"The Chemistry of Kanamycin" – M. Cron, O. Fardig, D. L. Johnson, F. Palermiti, H. Schmitz, and I. R. Hooper, Bristol Laboratories Inc., Syracuse, N. Y.

"Microbiological Studies with Kanamycin" – A. Gourevitch, V. Rossomano, T. Puglisi, J. Tynda, and J. Lein, Bristol Laboratories Inc., Syracuse, N. Y.

Discussant: W. E. Clapper, Lovelace Clinic, Albuquerque, New Mexico.

"Pharmacological Studies with Kanamycin" – D. E. Tisch, J. B. Huftalen, and H. L. Dickison, Bristol Laboratories Inc., Syracuse, N. Y.

"*In Vitro* and Pharmacological Studies with Kanamycin" – Henry Welch and W. W. Wright, Division of Antibiotics, U. S. Food and Drug Administration, Washington, D. C.

"Kanamycin Treatment of Experimental Infections in Mice" – G. A. Hunt and A. J. Moses, Bristol Laboratories Inc., Syracuse, N. Y.

"Effects of Kanamycin in Experimental Tuberculosis" – Ken Yanagisawa, Department of Tuberculosis, National Institute of Health, Tokyo, Japan.

"The Antituberculous Acitvity of Kanamycin *in Vitro* and in the Experimental Animal (Guinea Pig)" – William Steenken, V. Montalbine and J. R. Thurston, Trudeau Laboratories, Saranac Lake, N. Y.

General Discussion

Session Chairman: William B. Tucker
Central Office, Veterans Administration, Washington, D. C.

2:00 P.M. –
"Clinical Experiences with Kanamycin" – P. A. Bunn, A. Baltch, and O. Kraynjak, Department of Medicine, State University of New York College of Medicine, Syracuse, N. Y.

"Laboratory Studies with Kanamycin" – W. L. Hewitt and S. M. Finegold, University of California School of Medicine at Los Angeles, Los Angeles, Calif.

"Clinical Experience and Toxicity Studies with Kanamycin" – Fel G. Davies and Anthony Sainz, Marcy State Hospital, Marcy, N. Y.

"Kanamycin Serum Levels in Infants and Children" – Paul Wehrle and Sheldon Berger, Department of Pediatrics, State University of New York College of Medicine, Syracuse, N. Y.

"The Effect of Long-Term Kanamycin Therapy on Renal Function" – C. R. Kleeman, G. Chrisp, M. Winfield, and M. H. Maxwell, University of California School of Medicine at Los Angeles, Los Angeles, Calif.

"Case Report" – Leonard Berman and Sol Katz, Georgetown University School of Medicine, Washington, D. C.

Discussion

"Observations on the Use of Kanamycin, a New Antituberculous Agent in Man" – K. W. Wright, A. Renzetti, J. Lunn and Paul A. Bunn, Onondaga County Sanatorium and State University of New York College of Medicine, Syracuse, N. Y.

"Clinical Experience with Kanamycin in Chronic Pulmonary Tuberculosis" – J. H. McClement, J. F. Bowen, J. M. Jones, E. S. Nash, D. G. Simpson, and E. A. Riley, Chest Service, Bellevue Hospital, New York, N. Y.

"Treatment of Pulmonary Tuberculosis with Kanamycin" – Imasato Donomae, Governmental Committee of Chemotherapy for Tuberculosis and Medical Faculty, Osaka University, Osaka, Japan.

Discussants: R. L. Yeager, Summit Park Sanatorium, Pomona, N. Y.
J. W. Raleigh, Veterans Administration Hospital, Sunmount, N. Y.

FRIDAY, JULY 11, 1958

Session Chairman: Paul A. Bunn

Onondaga County Sanitorium and State University of New York College of Medicine, Syracuse, N. Y.

9:00 A.M. –

"The Effect of Kanamycin on Neurological and Electroencephalographic Abnormalities in Hepatic Failure" – T. C. Chalmers, K. Sebestyen, and W. H. Timberlake, Lemuel Shattuck Hospital, Boston, Mass.

"The Effect of Kanamycin on Blood Ammonia Levels in Cirrhosis" – W. W. Faloon and C. J. Fisher, Department of Medicine, State University of New York College of Medicine, Syracuse, N. Y.

"Intraperitoneal Use of Kanamycin as an Adjunct in the Therapy of Established Peritonitis and Peritoneal Contamination" – A. Prigot, B. Shidlovsky, and E. A. Campbell, Harlem Hospital, New York, N. Y.

"Kanamycin for Bowel Sterilization" – Isidore Cohn, Jr., Louisiana State University College of Medicine, New Orleans, La.

"Kanamycin Experience in Selected Cases" – K. J. R. Wightman, Department of Therapeutics, University of Toronto College of Medicine, Toronto, Canada.

General Discussion: Introduced by Saul Blau, New York University Post-Graduate Medical School, New York, N. Y.

"Some Experiences with Kanamycin in the Treatment of Salmonella and Shigella Infections" – R. V. Platou and W. G. Thurman, Department of Pediatrics, Tulane University School of Medicine, New Orleans, La.

"Laboratory and Clinical Experiences with Kanamycin" – F. Ruiz Sanchez and A. Ruiz Sanchez, Institute of Pathology and Infectious Diseases, University of Guadalajara, Jalisco, Mexico.

"Kanamycin Treatment of Urinary Infections" – Tokuji Ichikawa, Tokyo University School of Medicine, Tokyo, Japan.

"Kanamycin in the Treatment of Chronic Urinary Tract Infections" – A. H. Dube, Van Duyn County Memorial Hospital and State University of New York College of Medicine, Syracuse, N. Y.

"Kanamycin in Urologic Practice: Preliminary Observations" – R. D. Herrold, University of Illinois School of Medicine, Chicago, Ill.

General Discussion

Session Chairman: Edwin J. Pulaski

Department of Surgery, Letterman Army Hospital, San Francisco, Calif.

2:00 P.M. –

"The Staphylococcal Problem" – Vernon Knight, Vanderbilt University School of Medicine, Nashville, Tenn.

"Clinical and Laboratory Studies with Kanamycin" – A. C. White and Vernon Knight, Vanderbilt University School of Medicine, Nashville, Tenn.

"Kanamycin in the Treatment of Infections in Infants and Children" – R. H. High, A. Serria, and N. H. Huang, Department of Pediatrics, Temple University School of Medicine, Philadelphia, Pa.

"Clinical and Laboratory Studies with Kanamycin" – G. A. Cronk and D. E. Naumann, Department of Health and Preventive Medicine, Syracuse University, Syracuse, N. Y.

"Clinical Experience with Kanamycin" – S. M. Finegold, L. Guze, M. Winfield, and W. L. Hewitt, University of California School of Medicine at Los Angeles, Los Angeles, Calif.

"The Efficacy of Kanamycin in the Treatment of Surgical Infections" – A. M. Rutenburg, G. Koota, and F. Schweinburg, Departments of Surgery, Beth Israel Hospital and Harvard Medical School, Boston, Mass.

"The Use of Kanamycin in a Staphylococcal Epidemic in Children" – Martha Yow and G. Kenneth Womack, Department of Pediatrics, Baylor University College of Medicine, Houston, Texas.

"Clinical and Laboratory Evaluation of Kanamycin in Resistant Bacterial Infections" – E. M. Yow and O. Monzon, Department of Medicine, Baylor University College of Medicine, Houston, Texas.

Discussant: William L. Hewitt, University of California School of Medicine at Los Angeles, Los Angeles, Calif.

Summary of Conference: Maxwell Finland

NEW MEMBERS

Elected May 22, 1958

LIFE MEMBERSHIP

Cumming, John W., General Biology. Executive Vice-President, Carworth Farms, Inc., New City, N. Y.

SUSTAINING MEMBERSHIP

Abbate, Grace McLean, M.D., Psychoanalysis, Private Practice, Brooklyn, N. Y.
Allen, William H., Ch.E., Industrial Chemistry. Assistant Chief Engineer, Food Machinery and Chemical Corporation, Bronxville, N. Y.
Brady, John P., M.D., Sterility and Steroids. General Practice, Tillamook, Oreg.
Coughlin, Paul J., M.D., Biology and Chemistry. Private Practice, Tallahassee, Fla.
Crawford, R. L., M.D., Medicine. General Practice, Lancaster, S. C.
Daniel, Cuthbert, M.S., Private Consultant, New York, N. Y.
Diller, Alford C., M.D., Clinical Medicine. General Practitioner, Convoy, Ohio.
Dodge, George A., M.D., Internal Medicine. Private Practice, Portsmouth, N. H.
Duany, Emir V., M.D., Cardiopulmonary Physiology. Attending Comprehensive Courses, New York Polyclinic Medical School and Hospital, New York, N. Y.
Ducey, James Robert, Jr., Ph.D., Engineering. Director, Research and Development, East Orange, N. J.
Edwards, Lonnie C., M.D., General Surgery. Private Practice, Medicine, Chicago, Ill.
Fineberg, Henry H., M.D., Psychology. Private Practice, Winnetka, Ill.
Fochios, Steve E., M.D., Psychoanalysis. Senior Psychiatrist, Rockland State Hospital, Orangeburg, N. Y.
Hightower, George L., M.D., General Practice. Practicing Physician, Atlanta, Ga.
Hutschnecker, Arnold A., M.D., Psychology. Practicing Physician, New York, N. Y.
McLaughlin, Henry M., M.D., Cardiovascular Diseases. Private Practice, Lynwood, Calif.
Millender, Charles White, M.D., Medicine. Surgery, Memorial Mission Hospital, Asheville, N. C.
Morgan, Henry Gordon, M.D., Comparative Anatomy, Taxidermy. Private Practice—Dermatology, Los Angeles, Calif.
Sarnecki, M. M., M.D., Surgery and Metabolic Diseases. Private Practice, St. Paul, Minn.
Smith, C. Conrad, M.D., Dermatology. Clinical Instructor, Medical College of Georgia, Augusta, Ga.
Sorkin, Reuben, M.D., Medicine. Washington Highlands Medical Clinic, Washington, D. C.
Weary, W. B., M.D., Neurological Surgery. Private Practice, Dallas, Texas.

ACTIVE MEMBERSHIP

Abrahams, Irwin Willard, M.D., Ophthalmology. Intern—Medicine, Johns Hopkins Hospital, Baltimore, Md.
Abrams, Archie A., M.D., Obstetrics and Gynecology. Associate Professor, Boston University School of Medicine, Boston, Mass.
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